STUDIES OF WIREWORM POPULATIONS

II. SPATIAL DISTRIBUTION

BY GEORGE SALT, SC.D. (*King's College*) AND
F. S. J. HOLLICK, PH.D. (*St John's College*)

From the Department of Zoology, Cambridge

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(With Four Text-figures)

INTRODUCTION

The distribution of wireworms in space is known to be not entirely haphazard. It can therefore be assumed to be dependent upon a number of environmental factors, some favourable others unfavourable, the balance of which, varying from place to place, checks the pest here, but there allows it to occur in abundance. A thorough knowledge of those environmental factors would be of great value to the economic entomologist. It would enable him to estimate, without laborious sampling, the number of wireworms likely to be found under any given set of conditions. It might enable him to hinder the favourable and assist the unfavourable factors, and so to bring about control.

One way to obtain that desirable knowledge is to choose an environmental factor arbitrarily, and study it in detail in the laboratory, with hope that the information obtained will be of use. Another way is to examine the distribution of wireworms in nature, with a view to the discovery of factors correlated with the degree of infestation.

It is the second course that has been followed in the work to be described below. The distribution of wireworms in natural pastures has been studied in large, medium and small areas; first, to discover how wireworms are in fact distributed, and secondly, in the hope that many environmental factors could be eliminated, and a few shown to be important in determining the incidence of wireworms in those areas. This study, therefore, is not an attempt to investigate exhaustively the factors that control wireworm distribution, but an exploration to discover what factors are most likely to repay detailed investigation.

The method used to extract the wireworms from soil samples has been described in the first paper of this series (Salt & Hollick, 1944). It is considered to give the complete *Agriotes* population of the sample other than eggs.

FIELD DISTRIBUTION

A. *Spinney Pasture*

The distribution of wireworms within a grass field has been studied on the University Farm, 2 miles north-west of Cambridge, in a field of about 8 acres, called Spinney Pasture. This field is contiguous over part of its north-western boundary

with a small spinney of larch, fir, hornbeam and other trees, and abuts on the south upon a narrow belt of deciduous trees, almost exclusively ash. Apart from those woods, the pasture is surrounded by arable land; but the fields to the south-east were ploughed from very old grass as recently as 1940 and 1941, and those to the north and west have included in their rotation a 4-year ley. From the fields to the north-east and south-east, the pasture is separated by deep ditches. Spinney Pasture has been under grass for many years but was ploughed and resown to grass in 1933 when it was taken into the University Farm. Since that time it has been continuously under grass and principally used for grazing, though it has occasionally been cut for hay.

Twenty stations were established in the main area of the field in the positions shown on the map (Fig. 1). At each station, a sample was taken in the last week of every month from January 1942 until April 1944, when the field ceased to be available to us. Sets of samples taken from the field in October and December 1941 are not included in the following account, because they were not taken at the same stations; but a special set taken on 13 March 1944 is included. We have thus a series of twenty-nine samples from each station.

The samples collected on each occasion consisted of a cylinder of soil, 4 in. in diameter and 12 in. deep. The soil was collected and examined in two parts, an upper core from the surface to 6 in. deep and a lower core from 6 to 12 in. deep. The position of the samples relative to each station is shown by the diagram on the map (Fig. 1). Had it been possible at the outset to foresee that our work would be continued, the position of the samples might have been randomized; as it is, those of 1942 are more closely grouped about the station point than those of 1943 and 1944. The holes made by the removal of the samples were immediately filled with soil brought from the edge of the field and were capped with turf, so as to interfere as little as possible with the wireworm population and its environment. Since one 4 in. sample was taken in each square yard, just under 1 % of the soil was abstracted from the sampling area about each station.

The wireworm collections made in Spinney Pasture are composed almost exclusively of *Agriotes sputator*. In the whole course of our sampling of this field, we have collected 440 adult *Agriotes*, of which 437 were of that species. We have also identified 2430 larvae, using the character provided by Guéniat (1934), and found 2406 of them *sputator*. Of the 2870 identified *Agriotes*, then, 2843, or just over 99 %, were *A. sputator*. This existence of a nearly pure population of *A. sputator* is interesting, especially in view of the fact that in the field known as Trinity 1, only 200 yd. to the south-east, the *Agriotes* population is mixed and other species make up more than half of it.

B. *Distribution of wireworms at Spinney Pasture*

The basic data for the following study of field distribution, the number of wireworms recovered from each station at each of the twenty-nine collections made between January 1942 and April 1944, are recorded in Table 1.

Analysis of these data shows a significant variance with regard both to the months

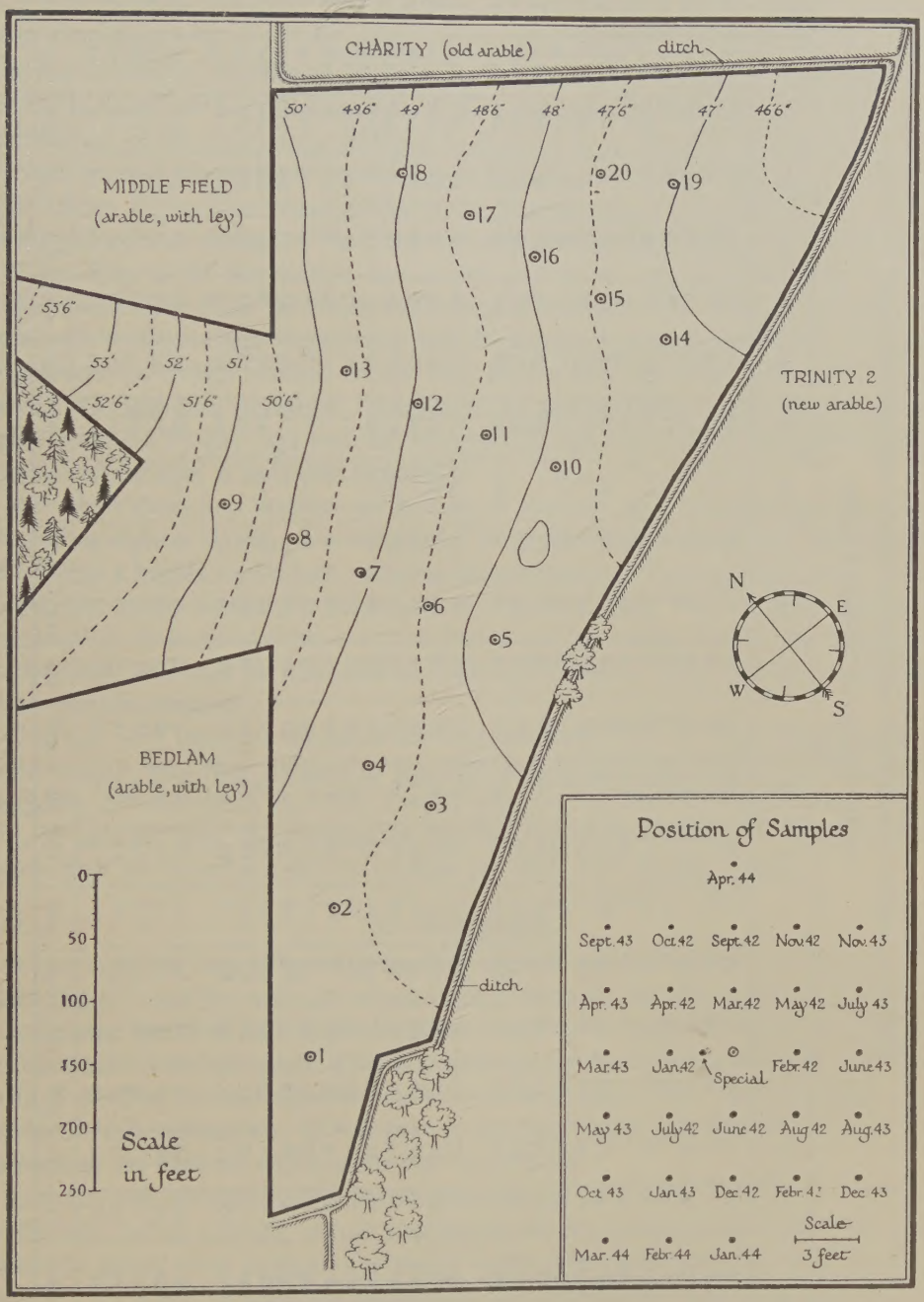


Fig. 1. Map of Spinney Pasture, showing the positions of the twenty stations and (inset) the positions of the samples taken at each station.

Table 1. *Number of wireworms collected on twenty-nine occasions from twenty stations at Spinney Pasture*

Station ...		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Month total
1942	Jan.	2	6	10	15	24	7	17	25	15	11	13	32	1	24	15	8	23	11	10	21	290
	Feb.	0	11	8	19	24	18	16	13	26	12	21	9	12	11	12	6	19	9	22	31	299
	Mar.	0	10	31	25	36	5	13	15	13	12	9	12	4	21	17	9	23	15	12	16	298
	Apr.	0	5	15	15	3	14	6	15	7	8	8	6	6	11	7	13	19	9	8	15	190
	May	1	21	8	11	13	14	17	8	18	8	13	17	12	9	16	6	20	4	16	10	242
	June	0	7	11	0	8	8	26	10	34	19	14	10	1	21	9	5	15	4	5	9	216
	July	0	18	24	9	34	38	10	13	17	20	9	27	20	13	18	10	21	9	15	83	408
	Aug.	0	12	12	6	20	7	23	6	21	30	20	9	8	12	43	14	19	29	30	33	354
	Sept.	4	4	34	11	2	15	15	8	11	7	14	37	5	12	6	3	18	30	21	15	272
	Oct.	5	14	25	32	24	28	44	8	18	9	16	23	1	24	10	9	26	28	22	24	390
	Nov.	4	33	34	32	41	29	25	12	11	7	43	21	3	35	9	16	30	18	26	22	451
	Dec.	1	9	19	17	25	39	20	25	16	41	15	9	21	33	28	28	18	46	26	34	470
1943	Jan.	1	23	5	15	24	9	15	20	13	17	19	22	22	8	7	12	18	23	30	30	333
	Feb.	0	32	5	20	19	21	18	24	20	25	22	11	18	22	27	11	15	15	19	26	370
	Mar.	1	23	16	12	16	14	22	9	18	29	38	10	4	38	31	8	26	31	10	38	394
	Apr.	2	9	30	21	43	18	19	18	7	10	27	20	12	23	26	29	14	9	28	12	377
	May	2	12	13	17	35	39	14	4	19	17	28	15	15	21	10	21	19	8	16	25	350
	June	7	7	23	13	12	11	8	13	20	18	10	7	5	7	9	11	11	13	13	15	233
	July	3	4	7	27	8	17	20	11	19	10	18	1	5	3	9	19	8	11	6	18	224
	Aug.	4	10	23	22	22	11	11	10	10	9	16	1	4	6	8	13	9	7	19	14	229
	Sept.	4	18	22	7	12	16	31	11	11	21	30	21	6	8	23	14	19	29	16	37	356
	Oct.	6	11	13	23	25	20	10	19	2	17	25	10	14	44	16	9	24	19	26	30	363
	Nov.	1	15	21	14	27	24	21	29	21	18	25	14	8	21	38	25	37	26	27	59	471
	Dec.	6	11	15	20	24	9	12	11	17	24	21	13	8	12	37	14	15	18	19	24	330
1944	Jan.	0	22	11	18	35	32	13	17	17	13	23	10	14	30	23	19	13	37	19	29	395
	Feb.	0	28	33	35	12	14	21	10	26	25	49	7	8	33	20	25	15	22	33	34	450
	Spec.	4	13	5	16	27	18	24	4	16	21	31	21	2	34	5	22	37	19	18	38	375
	Mar.	3	13	18	35	38	8	12	17	6	12	19	7	2	14	29	24	21	22	28	16	344
	Apr.	2	10	17	23	7	17	14	5	29	16	24	12	5	19	29	27	20	17	23	18	334
Station total		63	411	508	530	640	520	517	390	478	486	620	414	246	569	537	430	572	538	563	776	9808

of the year and to the stations of the field. The matter of seasonal occurrence will be dealt with in another paper. The spatial distribution, our present concern, shows so great a variance ($F = 10{\cdot}9$; 1% point 1·94) that it can be concluded with certainty that the wireworm population of Spinney Pasture is not distributed at random.

This is fortunate for the purposes of the present study, because, in so far as the distribution of the wireworms in Spinney Pasture is not random, it must be due to the operation of one or more controlling influences. The field therefore provides an opportunity for further investigation of the distribution of wireworms in space, an investigation, that is, bent on the discovery of the factors controlling the distribution.

It has first to be determined how many and which stations among the twenty differ significantly from the mean infestation of the field. The mean total collection from each station is 490 wireworms, and the standard deviation of the total collections from the twenty stations is 148. The total collections from only four stations exceed that standard deviation. Stations 1, 13 and 20, from which the collections differ from 490 by 427, 244 and 286 respectively, are well outside the limit; station 5, differing by 150, is only just beyond it.

But when these four stations are left out of account, and the collections from the remaining sixteen stations are submitted to an analysis of variance, that analysis still shows a highly significant variance among stations ($F = 2{\cdot}35$; 1% point 2·08). Therefore, while special attention can be focused upon four stations markedly abnormal in their infestation, the distribution of the wireworms in the remainder of the field may still be expected to yield information about factors controlling wireworm distribution.

Our task now becomes the quest for environmental factors which might explain, in particular, why four stations in the field should have wireworm populations so markedly different from the other sixteen stations, two very high and two very low, and, in general, why the wireworms are distributed in the field as they are. The factors that we have been able to explore are dealt with serially below.

C. *Topography*

A glance at the map (Fig. 1) shows that there is no local association between the two stations, 1 and 13, with low populations or between the two, 5 and 20, with high populations. Stations with higher or lower populations, respectively, come between the members of the two pairs. This indicates that we have to deal with four different parts of the field, not merely with two stations in a region of low and two others in a region of high infestation. It has first to be investigated whether anything in the position of the stations will explain their infestation.

Position with reference to the surrounding fields will not do. Station 20 is nearest to the field called Charity, across a ditch to the north-east, which has been continuously arable since 1933 and probably longer, and in which the examination of twenty 4 in. samples taken to a depth of 12 in. yielded only three wireworms. Station 20 has the highest infestation. Station 1 is equally near to Bedlam, the field on the west which, although including a 4-year ley in its rotation, also has a very low wireworm content (seven wireworms in fifteen 4 in. samples taken to a depth of

12 in.). Station 1 has the lowest infestation. The other stations near Charity, 14–18 and especially 19, and those near to Bedlam, 2 and 4, are not especially heavily or lightly infested. It does not appear that either a low or a high population in different parts of Spinney Pasture can be attributed to the influence of adjacent fields.

Station 1 is sufficiently close to the narrow strip of woodland on the south for a shadow to be cast on it rather late in the morning during the winter. Frost therefore lies later in the day on this station than on any other in the field. But its small wireworm population can scarcely be attributed to this factor, for the other station with low infestation, station 13, is completely without shade and one of the most exposed on the field.

Altitude is a topographical feature that can be more precisely investigated. Through the kindness of Dr R. D. Davies and Mr T. R. C. Fox, of the Engineering Laboratory, Cambridge, we were able to make a detailed survey of Spinney Pasture. Six-inch contours are shown on the map (Fig. 1), and the exact station levels are included for reference in Table 2. A comparison of the wireworm infestation and the altitude of all stations shows a significant correlation ($r = -0{\cdot}455$; 5% point 0·444). Some attention is therefore attracted to the position of the stations with regard to their altitude.

Finally, the position of the stations has to be considered in relation to the use of the field. For instance, in the summer of 1942, sow pens were placed in the southerly part, in the region of stations 2–13, but not about stations 1 or 14–20. We have not a sufficiently detailed history of the field to consider such factors directly, but their influence will be reflected in the soil analyses and in the flora and fauna. The effect of carting through part of the field is considered below in the paragraphs on soil consolidation.

D. *Soil*

(1) *Soil profile*

The soil of Spinney Pasture is a sticky, tawny-yellow clay derived from the gault (cf. Nicholson & Hanley, 1936, pp. 34–41). Below the mat of grass roots is a layer 7 or 8 in. thick where the clay is mixed with humus and appears dark grey in colour. When it dries this grey soil becomes crumbly and forms small, hard lumps very difficult to break down in the sieves. This layer and the root layer together occupy the upper 10 or 11 in. of soil and represent the region of cultivation. Beneath is the yellowish clay subsoil, in some places very sharply demarcated, elsewhere separated by a transition zone of 1 or 2 in. where the yellow clay merges gradually into the grey upper region. The yellow clay is dusted and speckled in places with calcium carbonate, and it also contains sparse, usually small, flints and pebbles. Small fibrous roots penetrate the clay at least as deep as 24 in. and leave minute pores and crevices in which small arthropods can be found. The parent gault (horizon 2 of Nicholson & Hanley, 1936, p. 35) begins to be apparent in Spinney Pasture at an average depth of 26 in. and is nine-tenths pure blue gault at an average depth of 32 in. At three stations, 14, 15 and 19, a layer of very distinctive orange-coloured sandy clay lies over the gault.

The depths of the three horizons—the beginning of the tawny-yellow clay, the first appearance of the blue gault, and the surface of the nine-tenths pure gault—are recorded for each station in Table 2. Coefficients of correlation with the wireworm collections are given at the foot of the columns. None of these horizons appears to be significantly correlated with the wireworm infestation.

Table 2. *Particulars of the twenty stations at Spinney Pasture*

Station	No. of wireworms	Altitude in ft.	Soil profile Depth in in. of			Resistance of soil to Culpin probe, in lb., at			Soil moisture as % weight of dry soil at	
			Tawny clay	Blue clay	Nine-tenths gault	1–4 in.	5–8 in.	9–12 in.	3 in.	9 in.
1	63	48·8	11	32	34	29·1	26·3	28·0	35·2	39·1
2	411	48·7	9	33	37	37·3	28·9	23·4	34·7	36·2
3	508	48·3	10	30	34	33·6	23·3	24·2	33·9	37·2
4	530	48·7	8	28	34	33·1	22·9	22·6	34·8	35·1
5	640	48·0	10	22	32	26·8	21·7	20·9	33·0	36·2
6	520	48·5	7	23	30	31·6	27·1	28·3	33·5	34·1
7	517	49·1	7	20	28	29·9	21·3	21·3	34·3	32·9
8	390	49·9	10	22	28	29·3	23·4	22·8	33·5	34·0
9	478	50·8	8	23	32	25·4	19·6	20·7	35·2	35·6
10	486	47·7	10	30	39	28·9	22·3	22·9	31·2	28·8
11	620	48·4	9	22	30	23·8	18·1	21·1	32·1	33·2
12	414	48·9	9	23	32	29·3	21·8	22·1	32·7	35·4
13	246	49·6	9	27	31	33·1	21·4	20·1	32·7	33·4
14	569	47·1	11	34	39	27·9	22·1	22·7	30·6	32·5
15	537	47·4	9	19	27	34·6	22·0	26·6	31·3	29·1
16	430	48·0	12	26	33	28·3	23·3	20·3	31·4	32·6
17	572	48·3	9	26	31	23·8	19·6	21·9	32·3	34·0
18	538	49·0	13	25	30	34·3	22·4	20·9	34·4	35·4
19	563	47·2	10	25	27	25·4	22·5	25·5	32·2	31·2
20	776	47·5	12	25	28	26·2	23·5	22·8	33·2	32·8
r		−0·455*	0·036	−0·336	−0·234	−0·315	−0·352	−0·226	−0·303	−0·390

For 18 D.F. 5 % point is 0·444; 1 % point is 0·561. (* Significant.)

(2) *Soil consolidation*

A small amount of carting is done along the north-west side of Spinney Pasture. This raises the question whether the compression of the soil due to the weight of the carts might affect the wireworm population; and the question is apposite because the cart tracks usually pass near stations 1 and 13, both of which have a very low wireworm infestation.

Through the kindness of Mr Ronald Ede, of the School of Agriculture, Cambridge, we have been able to investigate this factor quantitatively. Mr Ede lent us an instrument designed and used by C. Culpin (1936) for measuring the resistance of soil to penetration by a steel probe. We took records with the Culpin probe in March 1944, after a period of dry weather. At most stations, four records were taken, at 18 in. north, south, east and west of the station point. At four stations, 1, 7, 13 and 20, four additional records were taken, $1\frac{1}{2}$ yd. in each cardinal direction from the station point. Each record was carried to a depth of 13 in. The instrument was calibrated against a weighing machine, and the recording (cf. Culpin, 1936, Fig. 2) was then read for each inch of depth.

A summary of the measurements is given in Table 2, where the means of the readings from 1 to 4 in. at each station are shown, and also those from 5 to 8 in., and from 9 to 12 in. The entries in the table are the means of thirty-two readings at each of stations 1, 7, 13 and 20; and of sixteen readings at each of the other stations.

All stations show a greater resistance to the probe in the upper 4 in., the zone of roots, than lower down; but the measurements at this level seem to bear little relation to the carting, for stations 3, 4, 15 and 18 are much consolidated though far from the carting that may possibly have affected stations 2 and 13. In any case, no correlation is indicated between consolidation at this level and wireworm infestation. Station 1 is very consolidated at the 9–12 in. level, but this can scarcely explain its low infestation, for station 6 exceeds it in respect of deep compression, and station 13, the other station with low wireworm infestation, has the lowest resistance at this depth of any station on the field. Coefficients of correlation for wireworm infestation and soil consolidation at each of the three levels are shown in Table 2. None is significant.

(3) *Soil moisture*

In March and April 1944 we thrice measured the moisture content of the soil at the twenty stations at Spinney Pasture. Immediately after the samples had been removed, a cork-borer was pushed into the wall of the hole so as to remove a small sample of earth about $\frac{3}{4}$ in. in diameter and $1\frac{1}{4}$ in. long. Two such samples were taken at a depth of 3 in. and were put together into a bottle. Similar samples were taken from the hole at a depth of 9 in. The soil moisture in these small samples was weighed and calculated as a percentage of the dry weight of soil. The means of the three measurements at 3 and at 9 in. at each station are recorded in Table 2.

We cannot claim to have explored, but merely to have glanced at, the possible relation between the wireworm infestation of Spinney Pasture and soil moisture. Of this variable factor, it would have been especially desirable to have taken measurements on each of the twenty-nine occasions when wireworm collections were made; and it is not surprising that our three measurements at each station show no significant correlation with the number of wireworms found there.

(4) *Soil analysis*

On the last three occasions when Spinney Pasture was sampled, in March and April 1944, duplicate samples were removed from immediately beside those examined for wireworms. The second samples were delivered, each as an upper and a lower core, to F. Hanley, M.A., of the School of Agriculture, Cambridge, who kindly arranged for analyses to be made of the soil. We are much indebted to Mr Hanley for his willing co-operation, without which this part of our paper could not have been written.

The analyses are summarized in Table 3, where each entry is the mean of the measurements from the three upper or the three lower cores, respectively, that were analysed from each station. At the bottom of each column is given the value of the coefficient of correlation between the means in that column and the total collection of wireworms from the corresponding station, as given in Table 1.

Table 3. *Analyses of the soil at each station at Spinney Pasture*

Upper soil, 0–6 in.; lower soil, 7–12 in. All analyses refer to air-dry soil.

Station	*p*H		Loss on ignition %		Organic carbon %		Nitrogen %		$CaCO_3$ %		K_2O parts per 10,000		P_2O_5 parts per 10,000	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
1	7·89	8·20	13·45	9·53	3·58	2·04	0·387	0·269	5·56	5·43	1·4	0·7	2·8	1·5
2	7·91	8·23	11·48	9·92	3·14	1·83	0·346	0·232	5·21	6·28	1·8	1·0	2·4	1·1
3	7·96	8·25	10·78	9·91	2·95	1·88	0·327	0·251	4·75	3·64	1·1	0·7	2·1	1·4
4	8·04	8·26	12·14	8·61	3·03	1·44	0·336	0·198	7·82	7·32	1·1	0·8	2·5	1·5
5	7·94	8·11	11·12	9·49	3·22	1·93	0·333	0·246	4·78	5·27	1·5	1·2	3·3	1·6
6	8·00	8·23	11·72	7·79	2·99	1·59	0·330	0·212	7·53	9·04	1·4	1·0	3·2	1·8
7	8·00	8·28	11·88	7·62	3·08	1·25	0·286	0·176	8·75	14·37	2·1	1·6	2·7	2·2
8	8·09	8·27	11·27	7·88	3·06	1·31	0·327	0·173	7·94	12·85	1·7	1·2	2·8	1·9
9	8·02	8·17	11·87	8·41	2·91	1·29	0·316	0·178	7·57	8·91	1·6	0·9	2·8	1·8
10	8·12	8·21	10·25	7·48	2·86	1·23	0·325	0·167	6·42	5·92	2·1	1·5	2·8	1·4
11	8·05	8·21	11·28	7·08	2·83	1·24	0·303	0·175	7·95	10·75	1·7	1·5	3·1	1·5
12	7·94	8·22	11·05	8·50	2·97	1·44	0·332	0·192	8·23	8·94	1·9	1·0	3·3	1·7
13	7·90	8·30	11·13	7·12	2·88	1·30	0·309	0·176	7·65	11·49	2·4	1·4	2·3	1·8
14	7·88	8·15	11·35	8·59	2·82	1·55	0·316	0·169	3·30	3·60	1·4	0·9	2·2	1·2
15	8·11	8·21	10·37	7·75	2·73	1·27	0·289	0·175	6·87	6·32	1·3	1·1	3·0	1·4
16	8·02	8·41	11·38	8·20	2·81	1·28	0·308	0·175	8·36	13·74	2·1	1·1	2·8	1·9
17	8·08	8·24	10·53	7·77	2·88	1·45	0·313	0·191	8·49	11·42	1·9	1·5	2·5	1·7
18	8·03	8·33	10·92	7·10	3·02	1·45	0·317	0·179	8·44	12·19	1·8	1·7	2·9	1·8
19	8·04	8·28	10·07	9·48	2·93	1·75	0·310	0·201	8·37	8·61	1·6	1·1	2·6	2·1
20	8·09	8·25	10·13	8·41	2·74	1·32	0·302	0·193	9·41	12·22	1·6	1·3	2·9	1·8
r	0·483*	−0·151	−0·626**	−0·111	−0·597**	−0·252	−0·606**	−0·283	0·186	0·087	−0·216	0·316	0·177	0·060

For 18 D.F. 5% point is 0·444; 1% point is 0·561. (* Significant. ** Highly significant.)

(*a*) *Hydrogen-ion concentration.* This was measured electrically, using a glass electrode. The average *p*H of the upper cores was 8·01, and of the lower cores 8·24. At no station did the *p*H differ markedly from these means (upper soil, $\sigma = 0{\cdot}076$; lower soil, $\sigma = 0{\cdot}065$). The coefficient of correlation of the wireworm infestation and the *p*H of the top cores, however, is 0·483, which is slightly higher than the value of r at the 5% point and suggests that there may be some relation between the two. No such relation can be based on the analysis of the lower cores, for which the coefficient of correlation is $-0{\cdot}151$.

(*b*) *Loss on ignition.* The entries in Table 3 represent the percentage loss in weight after ignition of the oven-dry soil, corrected for carbon dioxide from the carbonates but not, of course, for combined water. The mean loss of the upper cores from the twenty stations ($11{\cdot}2 \pm 0{\cdot}80\%$) and that of the lower cores ($8{\cdot}3 \pm 0{\cdot}92\%$) is a common value for old pastures and calls for no special comment. This factor affords some estimate of the amount of organic material present in the soil. If it is true that young wireworms can nourish themselves on decaying organic matter in the soil, as Langenbuch (1932, p. 295) has claimed, loss of weight on ignition might be expected to bear some relation to the wireworm infestation. For the upper cores, the coefficient of correlation between the wireworm infestation and loss on ignition at the twenty stations at Spinney Pasture is $-0{\cdot}626$, which is highly significant but, surprisingly, of a negative correlation. For the lower cores, the correlation ($-0{\cdot}111$) is non-significant.

(*c*) *Organic carbon.* The percentage of organic carbon in the samples was measured by Walkley's wet oxidation method. The mean value in the upper soil was $2{\cdot}97 \pm 0{\cdot}192\%$, and in the lower soil $1{\cdot}49 \pm 0{\cdot}259\%$. This factor, like the preceding, is related to the amount of organic matter in the soil and might, on general grounds, be considered likely to influence wireworm distribution. It is interesting, therefore, to find it related to the wireworm infestation in a manner very similar to the loss on ignition. The coefficient of correlation for the upper soil ($-0{\cdot}597$) is highly significant of a negative correlation; that of the lower soil ($-0{\cdot}252$) is non-significant.

(*d*) *Nitrogen.* This was determined by the Kjeldahl method. The mean for the upper soil at Spinney Pasture was $0{\cdot}321 \pm 0{\cdot}022\%$, and for the lower soil $0{\cdot}196 \pm 0{\cdot}030\%$. Since the nitrogen in the soil is derived largely from the organic matter present, it is not surprising to find that this factor falls into line with the two preceding in its relation to the wireworm infestation. The coefficient of correlation for the upper soil at the twenty stations ($-0{\cdot}606$) strongly suggests a relation between the nitrogen content and the wireworm infestation. That of the lower soil ($-0{\cdot}283$) is non-significant.

(*e*) *Calcium carbonate.* This was measured by means of a Collins calcimeter and is recorded in Table 3 as the percentage of $CaCO_3$ in the soil by weight. The mean $CaCO_3$ content of the upper soil at the twenty stations was $7{\cdot}17 \pm 1{\cdot}63\%$, and of the lower soil $8{\cdot}92 \pm 3{\cdot}35\%$. For neither the upper nor the lower soil does the coefficient of correlation, shown at the bottom of Table 3, suggest any relation between the wireworm infestation and $CaCO_3$ at this order of content.

(*f*) *Available potash.* The method used for estimating the available K_2O was a

modification of that proposed by Dyer. In this method the soil is treated with a 1% citric acid solution, using an additional quantity of citric acid equivalent to the carbonate present in the soil, and the percentage of K_2O in the extract is then determined. The measurements are recorded in Table 3 as parts per ten thousand by weight. The mean measurement for the upper soil was $1{\cdot}7 \pm 0{\cdot}35$, and for the lower soil $1{\cdot}2 \pm 0{\cdot}30$. The coefficients of correlation, shown at the foot of the appropriate columns of Table 3, do not support any suggestion that available potash in the amounts found at Spinney Pasture has any relation to the wireworm infestation.

(*g*) *Available phosphoric acid.* This was measured by treating the soil with a 1% citric acid solution, as described above for the estimation of available potash, and then determining the percentage of P_2O_5 in the extract. The mean proportion present in the upper soil from the twenty stations was $2{\cdot}8 \pm 0{\cdot}34$ parts per ten thousand by weight; and in the lower soil $1{\cdot}7 \pm 0{\cdot}28$ parts. The coefficients of correlation between the measurements of P_2O_5 and the wireworm infestation, shown at the bottom of Table 3, do not support any hypothesis of a relation between the two at Spinney Pasture.

E. *Vegetation*

Before taking a soil sample, we cut off the growing vegetation close to the surface of the ground with scissors, in order to avoid the needless examination of material. During that process it was a simple matter to note the different species of plants, and on twenty occasions we made a quantitative estimate of the vegetation covering each of our twenty samples. The following observations can therefore be regarded as based on 400 'quadrats' each of 12·6 sq.in.

In making our estimate, we assessed the percentage of the surface occupied by each plant species. The percentage area method has been criticized by West (1938) as inadequate for the analysis of the vegetation of pastures, but it would appear to be sufficient for our purpose, especially as our observations were made in every month of the year and therefore avoid much of the error due to seasonal foliation of the different species.

A summary of the vegetation at Spinney Pasture is shown in Table 4, where for each station there is recorded (1) the number of samples out of twenty on which the plant was present, and (2) the sum of the twenty estimates of the percentage area occupied. At the foot of each principal column is also recorded the coefficient of correlation between the entries in column (2) and the total collection of wireworms made at the corresponding stations.

Before the different plants and the twenty stations are considered separately, it may be noticed that, so far as the 400 samples represent the vegetation of the field as a whole, clover occupied 12% of the area, moss 12%, *Lolium perenne* 40%, *Agrostis stolonifera* 22%, *Dactylis glomerata* 8% and *Festuca ovina* 2%. Nearly 2% of the area was recorded as bare ground, and the remaining 2% was occupied by a miscellaneous flora.

(1) *Clover.* Under this name is included both *Trifolium pratense* and *T. repens.* Clover is generally distributed throughout the field. It was present on 232 of the 400 samples, and occupied 11·9% of their total area. The coefficient of correlation

between the area occupied at each station and the number of wireworms collected there does not indicate any correlation between the two.

(2) *Moss.* Dr P. W. Richards kindly identified specimens of moss from Spinney Pasture as *Brachythecium rutabulum*, but it is likely that other species not submitted to him are included in our estimates. Moss occurred at all stations but was present on only 176 of the 400 samples. It occupied a slightly larger total area than clover (12·2%), but was much less uniformly distributed. Its distribution, however, does not seem to be correlated with that of the wireworm infestation.

Table 4. *Summary of the vegetation at Spinney Pasture, based on the examination of twenty samples from each of the twenty stations*

In each column (1) shows the number of samples out of twenty on which that floral element was present; and (2) is the sum of the twenty estimates of the percentage area occupied.

Station	Clover		Moss		*Lolium*		*Agrostis*		*Dactylis*		*Festuca*		Bare ground		Other flora	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1	11	190	3	40	19	1435	4	90	3	50	2	45	2	50	2	100
2	10	205	12	485	19	1035	0	0	6	80	3	140	1	25	3	30
3	13	280	14	395	18	860	3	130	2	70	6	210	2	50	3	5
4	12	240	10	210	18	830	8	450	1	40	4	130	1	10	6	90
5	15	390	3	45	18	980	11	465	2	80	0	0	1	30	1	10
6	13	320	4	150	19	890	10	400	6	175	1	20	0	0	3	45
7	15	365	9	310	20	1080	4	190	4	55	0	0	0	0	0	0
8	10	150	8	190	17	1040	10	410	4	80	0	0	2	90	1	40
9	12	210	12	480	16	660	10	500	5	90	0	0	0	0	4	60
10	10	175	10	165	15	820	11	460	11	345	0	0	0	0	3	35
11	9	175	13	405	18	700	9	415	8	235	2	70	0	0	0	0
12	11	215	11	345	19	870	9	430	5	85	0	0	1	25	3	30
13	13	300	7	170	17	815	5	130	12	435	2	40	2	100	1	10
14	6	120	6	95	10	320	15	760	16	575	1	10	2	80	2	40
15	13	285	8	135	15	565	17	885	2	30	1	10	0	0	3	90
16	10	205	5	75	13	710	8	445	14	490	1	5	0	0	2	70
17	14	255	5	135	15	790	13	700	4	60	1	10	1	50	0	0
18	11	200	12	370	12	465	17	875	3	55	0	0	0	0	4	35
19	12	290	9	250	14	640	14	555	1	30	4	155	1	80	0	0
20	12	210	15	420	13	460	14	690	3	60	2	50	1	75	3	35
r	+0·155		+0·278		−0·651**		+0·590**		−0·118		+0·032		−0·154		−0·374	

For 18 D.F. 5% point is 0·444; 1% point is 0·561. (** Highly significant.)

(3) *Lolium perenne.* This is the dominant grass over the greater part of Spinney Pasture. It occurred on 325 of the 400 samples and occupied 39·9% of their total area. A marked correlation exists between our estimates of the area it covered on our samples from the twenty stations and the number of wireworms collected there, but the correlation is negative (−0·651).

(4) *Agrostis stolonifera.* This grass occurred on 192 of the 400 samples and occupied 22·5% of their total area, but it was less generally distributed than *Lolium*. At station 2 it did not appear on any of the twenty samples; and at stations 1, 3, 7 and 13 it was present on only a few samples and over a small area of them. The coefficient of correlation between the area it occupied on our samples and the wireworm collections from the twenty stations is highly significant (0·590) and suggests a positive relation between the two.

(5) *Dactylis glomerata.* Cock's-foot grass was recorded on 112 of the 400 samples and occupied 7·8% of their total area. It occurred at all stations but in such variable amounts that, although it covered at one station more space than *Lolium* and at three stations more than *Agrostis*, at five other stations it was found on only one or two samples of each twenty. Its distribution within Spinney Pasture does not show any correlation with the wireworm infestation.

(6) *Festuca ovina.* Only at four stations, 2, 3, 4 and 19, was sheep's fescue at all common; at nine other stations, small amounts were found on one or two samples; and at seven stations it did not occur. Over the whole area of the 400 samples, it occupied 2·2%. Our wireworm collections from the twenty stations show no correlation with the distribution of fescue.

(7) *Bare ground.* Small patches of bare ground, up to 6 or 7 sq.in. in extent, occurred on seventeen of the 400 samples. The area of bare ground shows no correlation with the wireworm collections.

(8) *Other flora.* In this column are included one or more observations of *Cnicus arvensis*, *Bellis*, *Taraxacum*, *Potentilla*, *Poterium* or *Ranunculus*, or of the grasses *Hordeum pratense*, *Trisetum flavescens*, *Poa* sp. or *Cynosurus cristatus*. These plants occurred on forty-four of the 400 samples and occupied 1·8% of their area. The distribution of this miscellaneous flora, treated as a whole, does not appear to be correlated with that of the wireworms at Spinney Pasture.

F. *Fauna*

To make a census of all the arthropod material from a standard soil sample is a laborious and time-consuming process in which we have only rarely been able to indulge. Even to collect and count the macroscopic elements of the fauna multiplies by three or four the time required for the removal of the wireworms alone. In 1942 we were unable to spare the time necessary for this extra work, and merely noted in general terms the presence of some of the larger arthropods. From February 1943, however, we collected certain groups of arthropods consistently, and noted the number of individuals present in each core. Those collections are summarized in Table 5. The entries in the table represent the total number of individuals collected at each station from the sixteen samples examined between February 1943 and April 1944.

The question will be asked, how complete are these collections of animals other than wireworms? Of the forms similar to *Agriotes* in size, it is likely that we collected all the individuals present in the samples. Tests made in the course of the development of our method (Salt & Hollick, 1944, p. 55) failed to recover additional specimens from the residues. Of the smaller forms listed in Table 5, the Staphylinidae and the Sciarinae, it is possible that our apparatus failed to recover some of the very young larvae. But on the occasions when we tried to collect the complete arthropod fauna, by adding to our apparatus sieves fine enough to retain the smallest Collembola and mites, we recovered no additional specimens of Staphylinidae and only two additional sciarine larvae. This gives us confidence that the kinds of animals listed in Table 5 were almost completely collected from the soil samples. It must

Table 5. *The numbers of some arthropods collected from sixteen samples from each of the twenty stations at Spinney Pasture*

Station	Chilopoda	Diplopoda	Spiders	Carabidae	Staphylinidae		Other Coleoptera		Ants		Parasitic Hymenoptera	Sciarinae	Other Nematocera
					Adults	Larvae	Adults	Larvae	Samples	Individuals			
1	10	1	11	3	36	28	7	30	0	0	4	116	46
2	40	7	7	6	31	8	7	18	1	10	6	25	22
3	35	33	5	5	20	13	7	25	1	1	8	19	10
4	80	34	17	10	88	11	13	9	1	1	7	103	9
5	88	33	30	16	75	18	11	23	5	739	10	2629	16
6	63	28	12	19	66	11	3	11	1	1	12	110	10
7	56	26	13	9	46	20	5	42	3	3	4	65	12
8	64	40	8	6	43	38	1	13	2	2	6	17	14
9	51	20	13	5	53	34	4	7	2	3	14	55	13
10	62	11	13	4	41	15	3	20	0	0	9	15	26
11	92	49	11	5	69	6	8	14	2	12	2	7	4
12	45	23	9	6	25	12	7	27	2	2	3	32	21
13	48	26	8	10	53	33	9	15	0	0	1	15	16
14	57	22	23	11	42	12	7	91	5	104	11	5	26
15	39	22	13	27	34	24	9	17	2	2	6	59	27
16	66	43	13	10	73	24	9	10	4	494	7	8	16
17	77	18	8	5	55	16	5	15	6	19	3	30	11
18	66	24	16	11	38	6	8	7	2	2	5	21	8
19	55	25	36	10	34	5	2	10	2	11	1	14	14
20	52	30	18	3	16	4	6	24	4	33	10	32	10
Total	1146	515	284	181	938	338	131	428		1439	129	3377	331
r	0·610**	0·445*	0·425	0·181	0·057	−0·597**	0·050		0·579**		0·316	0·221	−0·642**

For 18 D.F. 5% point is 0·444; 1% point is 0·561. (* Significant. ** Highly significant.)

be borne in mind, however, that the different habits of some of those animals may lead to a smaller proportion of their true population being present in the samples than is the case with wireworms. Many spiders living on the surface of the ground, for instance, probably made good their escape when the samples were being taken; while a much higher proportion of the diplopod population may habitually live below 12 in. deep and so have escaped collection.

(1) *Chilopoda.* These predaceous animals are said to feed principally on earthworms and soft-bodied arthropods. There seem to be few observations of their feeding habits in nature, and it is at least possible that wireworms are included in their diet. We collected a total of 1146 centipedes from the 320 samples. This number, giving an average of 3·6 per sample, is surprisingly high for such large predators. Their distribution at the twenty stations shows a highly significant correlation with that of the wireworms ($r = 0{\cdot}610$).

(2) *Diplopoda.* Although millipedes are not predaceous, we collected them as being common and conspicuous members of the soil fauna. A total of 515 were found in the 320 samples. The coefficient of correlation (0·445) of millipedes with wireworms at the twenty stations is just significant at the 5% point.

(3) *Spiders.* These are surface-dwellers rather than a part of the soil fauna, but they must be considered as agents possibly affecting wireworm distribution because they have been recorded as preying upon adult elaterids (Subklew, 1938, p. 539). The distribution of the 284 individuals we collected in Spinney Pasture was probably not correlated with the wireworm population, the coefficient of correlation, 0·425, being a little less than r at the 5% point.

(4) *Coleoptera.* Carabidae and Staphylinidae are listed separately in Table 5; the other families of Coleoptera are lumped together. Neither the predaceous carabids nor the Coleoptera of various habits seem to be connected with the wireworm distribution. Among the staphylinids, the adults show no correlation with the occurrence of wireworms; but the larvae, so far as our collections may be valid, are negatively correlated to a highly significant degree ($r = -0{\cdot}597$).

(5) *Hymenoptera.* Parasitic Hymenoptera, especially Proctotrypoidea, were collected, and also ants. The 129 parasites do not appear to be correlated with the incidence of the wireworms, but are perhaps too few for satisfactory demonstration. In the case of ants, some samples contained very many individuals, while others, though still within the territory of an ant colony, contained very few. We have therefore not used the number of individuals but rather the number of samples containing ants, as the measure of their distribution at the twenty stations. On this basis ants and wireworms occur together to a highly significant degree ($r = 0{\cdot}579$).

(6) *Diptera.* Only of Nematocera was a sufficient number of individuals collected to make their listing worth while. Of that group, the Sciarinae were most common and are recorded separately in Table 5. The Sciarinae gave an example of their curious gregarious habit in the occurrence of 2595 larvae in a single sample. Whether that sample is included or not, the Sciarinae do not seem to be concerned with wireworm distribution. The other Nematocera occurred more frequently where wireworms were few, the coefficient of correlation ($-0{\cdot}642$) being highly significant.

G. *Factors correlated with the wireworm distribution*

When we come to summarize the foregoing investigations, we find that of the forty-three correlation coefficients that have been calculated, twelve are significant. Nine of them are highly significant; those, namely, between wireworms and loss on ignition, organic carbon, nitrogen, *Lolium*, *Agrostis*, chilopods, staphylinid larvae, ants and Nematocera. The three that are significant only at the 5% point are the correlations between wireworms and altitude, *p*H, and diplopods. This list is long, but it can be reduced by further analysis.

The three highly significant correlations found between wireworms and nitrogen, organic carbon, and loss on ignition probably represent three observations of the same phenomenon. The nitrogen in the soil is derived largely from organic matter there present, and the amount of organic carbon and the loss on ignition are in large part measurements of the same material. Calculation of the partial correlation coefficients shows that, after eliminating the effects of the other two factors, the correlation between wireworms and loss on ignition is $-0{\cdot}348$, between wireworms and nitrogen $-0{\cdot}261$, and between wireworms and organic carbon $-0{\cdot}033$. It would appear, then, that in this complex of factors the greatest part of the correlation is due to that between wireworms and loss on ignition.

The next significant correlations to be considered are those with the grasses *Lolium* and *Agrostis*. The former is negatively, the latter positively, correlated with the wireworm infestation. Since the vegetation of our samples was measured as the percentage area occupied, so that where one floral element was abundant others were necessarily scanty, it may be that one of these two correlations is spurious and merely represents the reciprocal of the other. We do not know of any observations indicating that wireworms are attracted by *Agrostis* or repelled by *Lolium* and have no biological grounds for a choice between the two correlations. On statistical grounds, however, since the partial correlation coefficient between wireworms and *Agrostis* after adjustment for inequalities in *Lolium* is $0{\cdot}179$, while that between wireworms and *Lolium* after similar adjustment for *Agrostis* is $-0{\cdot}380$, we are led to suppose that the negative correlation between wireworms and *Lolium* is the more important.

Of the five factors so far considered, the two that claim most attention, loss on ignition and *Lolium*, are both negatively correlated with the incidence of wireworms. The two are correlated between themselves to a highly significant degree ($r = 0{\cdot}615$, with 18 D.F.). We should like to know whether the root system of *Lolium* is so abundant or so combustible as to control, in Spinney Pasture, the distribution of the loss on ignition, but we have not that information. What can be said is that the partial correlation coefficient of wireworms with *Lolium* in the absence of loss on ignition is $-0{\cdot}433$, that between wireworms and loss on ignition in the absence of *Lolium* is $-0{\cdot}377$. Also, that wireworms are known to occur in large numbers in black fen soil where the loss on ignition is much greater than the average of 11·2% found at Spinney Pasture. From these considerations it would appear more likely that the effective correlation is that between wireworms and *Lolium*, and that the

correlations between wireworms and the three measurements of organic matter result from it.

Even if that suggestion (it has no higher status) is correct, the correlation between wireworms and *Lolium* may still be secondary. It may be due, for instance, to the preference for a *Lolium* habitat of some predator which in that particular habitat reduces the wireworm population to a lower level than elsewhere.

Among the faunal elements two, chilopods and ants, are positively correlated with wireworms, and two, staphylinid larvae and Nematocera (other than Sciarinae) are negatively correlated. When we try to evaluate the significance of these correlations we are at once confronted with a question we cannot answer. Shall we expect the numbers of an effective predator to be positively or negatively correlated with wireworms in our collections or, indeed, will the numbers show any correlation at all? What little is known about the numerical interaction of predators and their prey (cf., for example, Lotka, 1925, and Gause, 1934) indicates that the numbers of a predator fluctuate after a lapse of time with those of the prey. But the information available has to do almost exclusively with the distribution of the two in time, especially over successive generations. Little of a quantitative nature is known about the relative distribution of the two in space; and nothing at all about their distribution in such a medium as the soil, where movement of the predator is impeded and where its perception of the prey must be limited to a relatively short distance. In looking for a possibly effective predator of wireworms in our collections, therefore, we cannot restrict ourselves to those members of the fauna for which we have found a significant correlation.

It is likely, however, that any predator that may control the wireworm distribution at Spinney Pasture is one that feeds on small wireworms. This is indicated by the characteristic size-composition of the wireworm population of the field. At Sheep's Green, the wireworm population can be represented by a roughly triangular histogram (Salt & Hollick, 1944, Figs. 4, 5). At Spinney Pasture, the population is composed of an excessive number of very small wireworms and a small and only gradually decreasing number of medium-sized and large wireworms (Salt & Hollick, 1944, Fig. 6). The great reduction in wireworm numbers at Spinney Pasture occurs just before the larvae reach a length of 6 mm. If a predator is concerned with this, it need not be a large animal and it may be expected to be a small one.

For this reason, we do not put much weight on the correlation between wireworms and chilopods. Their comparatively large size and their elaborate apparatus of poison fangs would be wasted on prey so much smaller than themselves. It may be of interest to record that at Spinney Pasture the distribution of chilopods is correlated to a highly significant degree with that of the diplopods ($r = 0{\cdot}637$, with 18 D.F.).

The positive correlation between the occurrence of ants and the incidence of the wireworm infestation at Spinney Pasture is supported by observations made at another field. There, a station situated in a region conspicuously populated by ants consistently gave high wireworm counts. The association, if it is real, must be an indirect one. Perhaps the presence of ants deters a predator.

The nematocerous larvae collected are almost entirely non-predaceous forms and cannot be supposed to affect the number of wireworms directly. We are rather surprised to find them negatively correlated with the wireworms. They are not significantly correlated with the occurrence of *Lolium* ($r = 0{\cdot}387$) or with loss on ignition ($r = 0{\cdot}367$, with 18 D.F.).

The final group that shows a highly significant correlation, the staphylinid larvae, includes many carnivorous and some parasitic forms. It might be supposed that they would be associated with dipterous larvae, but the coefficient of correlation between staphylinid larvae and Nematocera is 0·317, which is non-significant, whereas the partial correlation coefficient between wireworms and staphylinid larvae in the absence of Nematocera remains significant at $-0{\cdot}541$., Moreover, these staphylinid larvae are about the size that would be postulated for an arthropod predator attacking wireworms about 4–6 mm. long. These facts, statistical and biological, give some basis for an hypothesis that the correlation between staphylinid larvae and wireworms at Spinney Pasture is no coincidence, but an effective association.

One group, not included in Table 5, remains in our minds as possibly concerned in the destruction of small wireworms. That is the Acarina. Mites are extremely numerous in the soil of Spinney Pasture. We have already recorded finding the population to be at least 200 million acarines per acre (Salt & Hollick, 1944, p. 63), and more recent work, still to be published, shows that estimate to be far too low. We have repeatedly found mites on medium-sized and large wireworms. The role of mites in the wireworm environment deserves serious investigation.

The correlation coefficients between wireworms and altitude, *p*H, and diplopods are significant only at the 5% level of probability. That between wireworms and diplopods can be dismissed as of little immediate interest, since these animals are not predaceous. Wireworms occur in soils of such very different acidity and alkalinity that we cannot put much weight on the observed correlation within the comparatively narrow range of *p*H at our stations. The correlation with altitude suggests a study of the relation between altitude, soil moisture and wireworms, possibly with a floral link interpolated; but our measurements of soil moisture, as already explained, are regrettably inadequate.

It appears, then, that among the factors we have studied, those most clearly bound up with the wireworm distribution within Spinney Pasture are three: the amount of organic matter in the soil, the density of the grass *Lolium*, and the number of staphylinid larvae. Calculation of the partial correlation coefficients among the four variables—wireworms, loss on ignition, *Lolium* and staphylinid larvae—shows that, after eliminating the effects of the other two factors in each case, the coefficient of correlation between wireworms and loss on ignition is $-0{\cdot}335$, between wireworms and *Lolium* $-0{\cdot}388$, and between wireworms and staphylinid larvae $-0{\cdot}473$. Statistical analysis therefore points to the staphylinid larvae as the most important single factor we have studied; but we are not satisfied that our data are sufficient on biological grounds for that conclusion, and prefer to consider all three of these factors as being worthy of further study.

For the present, that is as far as we can carry our search for the factors that control wireworm distribution within a field. Later in this paper we shall give reasons to suggest that further progress in the analysis of the problem will be slow until other considerations have been added to the discussion, namely, those concerned with the composition of the wireworm population, rather than merely its size.

PLOT DISTRIBUTION

A. *Sheep's Green*

The distribution of wireworms within a plot has been studied on part of a field known as Sheep's Green, near the Fen Causeway, Cambridge. This is a low-lying common of about 7 acres, intersected by small streams and ditches, and sparsely planted with willows and Lombardy poplars. Some of the field is marshy, but most of it is under rough grass which is only lightly grazed and rarely, if ever, cut for hay. It is unlikely that Sheep's Green was ever under cultivation.

Our work there has been concentrated in an area of about $\frac{1}{4}$ acre near the centre of the field, bordered on one side by a brook and on others by a tarred path and a shallow ditch. On this area nine stations were arranged, 5 yd. apart, in a row running north and south; and seven stations, similarly spaced, in a nearly parallel row 15 yd. to the west (Fig. 2). At each station a sample was taken at the middle of every month from July 1941 until September 1942. Extra samples were taken in June, July and August 1942. After an interval of nearly 2 years, further samples were removed in May, June and July 1944. From each of the sixteen stations, then, we have examined twenty-one samples.

The samples taken on each occasion consisted of cylinders of soil, 4 in. in diameter and 12 in. deep. Each sample was removed and examined in two parts, an upper core from the surface to 6 in. deep and a lower core from 6 to 12 in. deep. The position of the samples relative to each station is shown by the diagram on the map (Fig. 2). Holes made by the removal of the samples were immediately filled with soil brought from an adjacent part of the field, and were capped with turf, so as to interfere as little as possible with the wireworm population and its environment. The primary samples were spaced a yard apart, but the extra samples and those taken in 1944 came between them so that, over the whole period of 3 years, 1·3% of the soil was removed from the immediate sampling area of 15 sq.yd. about each station.

The elaterid beetles collected from these samples include species of *Agriotes*, *Athous* and *Adrastus*, but in this paper, except for a paragraph on p. 29, we are concerned only with the *Agriotes*. Of that genus we have collected from Sheep's Green a total of 6921 individuals, among which there were fifty-one adults and 2542 larvae over 6·5 mm. long. All the adults and 2475 of these larger larvae have been identified; the other sixty-seven large larvae cannot be readily named because the critical parts are injured or missing. We have not identified larvae smaller than 6·5 mm. because it is difficult and takes longer to see the distinguishing characters in small larvae, but the wireworms above 6·5 mm. in length can be taken to be

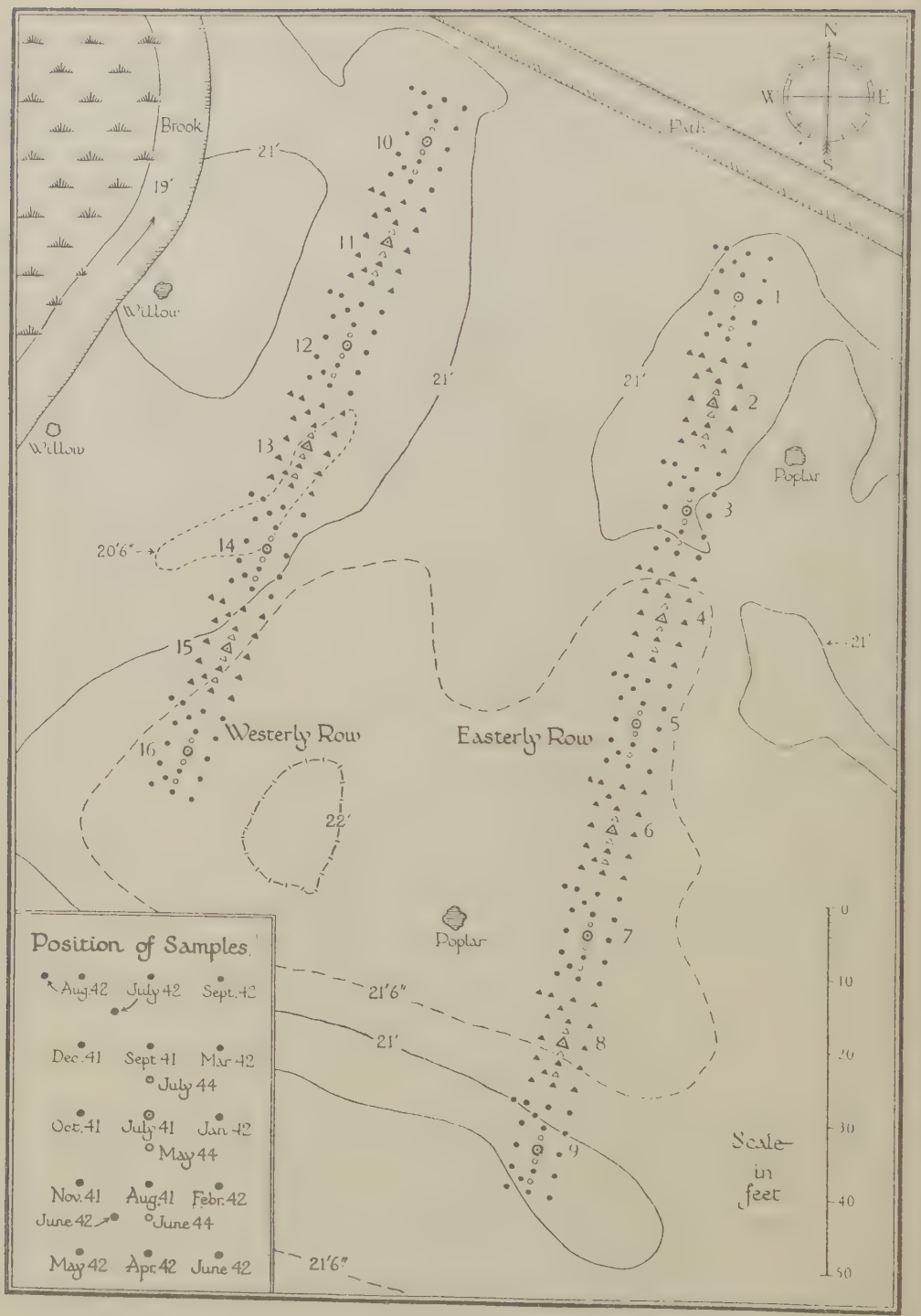


Fig. 2. Map of the plot of about ¼ acre at Sheep's Green, showing the points from which samples were taken.

sufficiently representative of the whole population. Of those identified, fifty adults and 2333 larvae were *A. sputator*, one adult was *A. obscurus*, and 142 larvae were *obscurus* or *lineatus*, which we have not attempted to distinguish in the larval stage. It appears from these figures that about 94 % of the *Agriotes* population of Sheep's Green is *A. sputator*. But the whole field is not uniformly infested with that species, and we shall have occasion later (p. 29) to show that *obscurus-lineatus* forms a high proportion of the *Agriotes* population at certain stations.

B. *Distribution of* Agriotes *at Sheep's Green*

More than half of our wireworm collections from Sheep's Green were obtained from square-yard and square-foot samples with which we are not here concerned. From the twenty-one 4 in. samples taken at each station, we collected a total of 2317 *Agriotes*, as set out in Table 6. These are the collections on which the present discussion of plot distribution is based.

Table 6. *Number of wireworms collected on twenty-one occasions from sixteen stations at Sheep's Green*

Station	Easterly row										Westerly row							
	1	2	3	4	5	6	7	8	9	Total	10	11	12	13	14	15	16	Total
1941 July	3	1	13	6	1	3	6	3	1	37	1	1	6	0	2	1	6	17
Aug.	4	16	16	10	6	5	12	1	1	71	3	1	3	1	6	3	3	20
Sept.	1	13	4	24	5	5	12	15	8	87	1	1	2	1	5	2	4	16
Oct.	4	9	3	24	19	4	16	19	6	104	3	4	8	1	0	0	12	28
Nov.	6	8	9	3	11	16	18	6	3	80	0	2	5	4	0	3	3	17
Dec.	0	9	14	22	3	9	25	3	6	91	2	3	2	2	0	1	73	83
1942 Jan.	3	1	4	16	5	14	51	26	0	120	3	0	1	3	0	4	5	16
Feb.	3	4	4	12	12	44	15	1	0	95	1	0	8	0	14	0	26	49
Mar.	2	4	36	25	3	2	12	19	1	104	4	1	2	1	1	1	21	31
Apr.	16	16	17	11	3	4	28	4	3	102	0	2	1	1	0	6	6	16
May	3	22	8	10	4	10	16	2	3	78	0	7	3	0	0	1	7	18
June	3	20	5	1	2	3	8	7	3	52	4	0	1	0	1	7	2	15
,,	2	15	8	7	3	11	12	5	0	63	1	4	3	1	1	2	12	24
July	1	9	11	29	10	2	35	9	0	106	1	2	4	1	2	3	8	21
,,	1	2	11	9	13	2	8	9	1	56	2	0	1	2	3	1	8	17
Aug.	1	18	4	16	5	5	7	14	4	74	2	0	3	3	2	0	84	94
,,	0	4	16	6	11	1	21	15	1	75	0	2	1	3	4	0	4	14
Sept.	0	10	7	16	3	12	9	3	5	65	8	0	2	1	0	4	3	18
1944 May	2	7	21	21	6	11	21	3	1	93	3	4	3	4	4	2	2	22
June	3	12	2	17	6	17	17	5	1	80	3	1	2	2	1	4	4	17
July	5	6	7	11	17	1	5	5	3	60	3	0	4	12	0	8	44	71
Total	63	206	220	296	148	181	354	174	51	1693	45	35	65	43	46	53	337	624

It requires no statistical treatment of the data of Table 6 to show that there are consistent differences between the sixteen stations at Sheep's Green in respect of their wireworm infestation. An analysis of variance of the data confirms that the variation between the means for the sixteen stations is far greater than that between different samplings at the same station ($F = 9{\cdot}21$; 1 % point 2·11), and thus forbids any hypothesis of random distribution

Inspection of the table also shows that throughout the period covered by these samples the stations forming the easterly row were twice as heavily infested as those forming the westerly row. But this cannot be taken to indicate that the area is simply divided into two parts, one of high infestation traversed by the easterly row, and one of low infestation traversed by the westerly row. Closer inspection shows that one station of the westerly row, 16, has a high infestation comparable with that of most stations in the easterly row; while the stations at the two ends of the easterly row, 1 and 9, each have a low infestation similar to most stations in the westerly row.

The sixteen stations therefore fall into two groups of eight. One group, comprising stations 2–8 and 16, includes most of the easterly row. This group provided 1916 wireworms; nearly 83% of the whole collection. These stations averaged 11·4 wireworms per sample, the standard deviation being 3·9. An analysis of variance on the 168 collections shows the difference between stations to be slightly greater than can be attributed to sampling variation, using a 5% level of significance. The other group, comprising stations 9–15 and 1, includes most of the westerly row. This group provided 401 wireworms, only 17% of the total. These eight stations averaged 2·4 wireworms per sample, with a standard deviation of 0·91. Analysis of the 168 collections from this group of stations gives no indication of a significant variation between stations as compared with the variation between separate collections from a station.

Not only is the difference between these two groups of stations very marked, it is also very persistent. It appears, as can be seen in Table 6, at the beginning of the sampling, in July 1941, and it is a regular feature of each collection until September 1942. Then, after a period of 19 months when no samples were taken, the difference reappears in the same order of magnitude in the collections of May, June and July 1944.

We can conclude that there is a marked and persistent non-random distribution of the wireworms within this experimental plot of about $\frac{1}{4}$ acre. It now lies before us to consider what factors might have brought about that distribution.

C. *Topography*

The immediate environs of our plot at Sheep's Green seem unlikely to affect the distribution of wireworms within the experimental area. The brook that runs near the westerly row (Fig. 2) has a steep eastern bank, and the surface of the water in it is usually about 2 ft. below the general level of the ground. The brook is balanced on the other side of the plot by a shallow ditch which lies just off the edge of our map. It is usually waterlogged, and carries running water in wet seasons. The position of the tarred path and of the trees is indiscriminate with respect to the rows of stations.

The plot itself has no marked features. The distance between the two rows is 15 yd., and between adjacent stations only 5 yd., so that larger wireworm larvae presumably, and the adults certainly, are able to move from one station to another. From general considerations it would seem unlikely that topographical features vary enough within a level plot of less than $\frac{1}{4}$ acre to have much influence on the distribution of wireworms.

One topographical feature at Sheep's Green, however, appears to belie that view. Six-inch contours, for the survey of which we are indebted to Mr T. R. C. Fox, are shown on the map. It is apparent that, on the whole, the westerly row passes across a shallow depression, while the easterly row runs over a plateau. Further than this, stations 1 and 9 lie in depressions, and station 16 on higher ground; so that the exact station altitudes, recorded in Table 7, show a highly significant correlation ($r = 0{\cdot}691$) with the total wireworm collection from each station. No one would suppose that wireworms are influenced by 6 in. of altitude as such, and it will be shown later that this correlation is one of several bound up with the soil at Sheep's Green.

Table 7. *Particulars of the sixteen stations at Sheep's Green*

Station	No. of wire-worms	Altitude in ft.	Depth of loam in in.	Resistance of soil to Culpin probe, in lb., at			Soil moisture as % weight of dry soil at	
				1–4 in.	5–8 in.	9–12 in.	3 in.	9 in.
1	63	20·9	17	23·3	26·9	30·4	30·3	25·9
2	206	20·6	17	19·3	19·6	22·7	31·8	28·7
3	220	20·8	18	23·8	23·2	24·4	23·6	24·8
4	296	21·5	22·5	23·1	23·8	27·0	26·8	22·6
5	148	21·6	25	24·1	25·7	29·7	27·5	25·6
6	181	21·7	25	26·8	34·3	37·9	26·1	22·9
7	354	21·8	25	21·9	29·3	30·4	23·1	21·0
8	174	21·7	24	25·0	30·2	35·2	25·6	22·8
9	51	20·9	18	21·9	27·6	27·9	29·2	22·3
10	45	20·6	8	22·9	21·2	21·9	36·7	38·2
11	35	20·6	9·5	21·3	21·9	21·7	36·7	36·2
12	65	20·5	9	20·8	19·2	21·3	36·1	35·5
13	43	20·4	8	20·8	19·7	20·5	39·9	37·3
14	46	20·5	7·5	17·2	20·3	24·8	38·1	30·1
15	53	21·3	24	22·8	22·9	23·8	27·8	22·5
16	337	21·6	25	22·4	27·9	30·3	24·5	21·8
r		0·691**	0·683**	0·257	0·441	0·450	−0·755**	−0·628**

For 14 D.F. 5 % point is 0·497; 1 % point is 0·623. (** Highly significant.)

D. *Soil*

(1) *Soil profile*

The soil at Sheep's Green is river alluvium and can be described as a shallow clay loam with heavy clay beneath. The loam is light brown in colour, very calcareous, and varies in depth from about 6 in. to at least 30 in. A mechanical analysis, kindly made for us by Mr Hanley, showed 12·8% coarse sand, 7·9% fine sand, 8·4% silt, 32·7% clay, 14·8% loss on ignition and 21·4% $CaCO_3$. The clay beneath is grey, mottled with reddish brown. Both layers contain many recent shells of which the most numerous are *Limnaea pereger* Müller and *Bithynia tentaculata* Linn.

The depth of the loam at each of the sixteen stations is given in Table 7. The considerable variation, from $7\frac{1}{2}$ to 25 in., is noteworthy. When the depth of the loam is considered with reference to the measurements of altitude, it appears that the clay underneath lies relatively level, and that the surface contours are principally due to the varying depth of loam lying on the clay table. Calculation shows that there is a highly significant correlation ($r = 0{\cdot}683$) between the depth of the loam and the number of wireworms at the sixteen stations.

(2) *Soil consolidation*

There is no carting across Sheep's Green and, although it is walked upon a good deal, there is no trodden path across our experimental plot. Measurements of the soil consolidation were made with the Culpin probe (see p. 7, above) 1 ft. north, south, east and west of each station point, and the records were read for each inch of depth to 12 in. There were therefore forty-eight readings at each station. The means of the sixteen readings from 1 to 4 in. at each station are given in Table 7, as also are those from 5 to 8 in., and from 9 to 12 in.

The mean resistance at all depths (24·6 lb.) was almost exactly the same as at Spinney Pasture, but, unlike that field, where the surface layer was most consolidated, the soil at most stations at Sheep's Green provided a gradually increasing resistance as the probe went down. Coefficients of correlation between the resistance at the three depths and the wireworm population at each station are recorded at the bottom of the table. None is significant.

(3) *Soil moisture*

The amount of moisture in the soil at Sheep's Green was measured in connexion with the samples taken in May, June and July 1944. The measurements were made by the same method as that used at Spinney Pasture (p. 8), and the weight of soil moisture was calculated as a percentage of the dry weight of soil. Means of the three measurements at 3 in. depth at each station are recorded in Table 7, as also are those at 9 in. depth.

At both depths the mean measurements show a highly significant correlation with the number of wireworms collected. The coefficient of correlation between wireworms and soil moisture at 3 in. depth is $-0{\cdot}755$; at 9 in. depth $-0{\cdot}628$.

(4) *Soil analysis*

On the last three occasions when Sheep's Green was sampled, in May, June and July 1944, duplicate samples were removed from immediately beside those examined for wireworms. They were delivered to Mr Hanley at the School of Agriculture, Cambridge, who very kindly arranged for analyses to be made of the soil. The methods used in this case were the same as those used for the soil of Spinney Pasture, and need not be detailed again. The results are summarized in Table 8, where each entry is the mean of the measurements of the three upper or the three lower cores, respectively, from each station.

(*a*) *Hydrogen-ion concentration.* The average *p*H of the upper cores was $7{\cdot}98 \pm 0{\cdot}04$, and of the lower cores $8{\cdot}31 \pm 0{\cdot}04$. The coefficients of correlation between the total number of wireworms collected at each station and the *p*H of the upper and of the lower cores are 0·029 and 0·040 respectively. Wireworm distribution does not appear to be correlated with *p*H within this range.

(*b*) *Loss on ignition.* The mean loss on ignition of the upper cores from the sixteen stations was $14{\cdot}8 \pm 1{\cdot}4\%$, which is 3·6% more than at Spinney Pasture. Of the lower cores it was $8{\cdot}2 \pm 0{\cdot}6\%$, practically the same as at the other field. The

Table 8. *Analyses of the soil at each station at Sheep's Green*

Upper soil, 0–6 in.; lower soil, 7–12 in. All analyses refer to air-dry soil.

Station	*p*H		Loss on ignition %		Organic carbon %		Nitrogen %		$CaCO_3$ %		K_2O parts per 10,000		P_2O_5 parts per 10,000	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
1	8·02	8·36	16·63	9·00	6·94	2·78	0·702	0·342	24·54	30·95	1·0	1·0	8·0	3·5
2	8·07	8·28	13·84	9·43	5·87	3·17	0·604	0·390	23·48	28·74	1·7	0·9	5·8	6·1
3	7·99	8·36	11·93	7·46	5·04	2·25	0·548	0·289	24·42	28·07	1·3	0·5	8·1	5·8
4	7·96	8·28	13·13	8·67	6·52	2·68	0·676	0·344	19·54	24·96	0·8	0·9	6·1	5·1
5	7·98	8·36	16·19	9·04	6·47	2·72	0·681	0·350	20·02	24·91	1·8	0·9	12·5	5·9
6	8·00	8·33	15·96	8·18	6·64	2·65	0·693	0·349	18·41	25·24	1·5	0·7	9·0	6·6
7	7·99	8·31	15·51	8·04	6·05	2·64	0·635	0·333	17·31	25·53	0·9	0·7	5·7	6·7
8	7·89	8·32	14·43	7·78	6·46	2·57	0·649	0·325	16·79	23·81	0·9	0·8	7·4	6·3
9	7·92	8·26	14·90	8·39	6·40	2·47	0·658	0·318	26·87	28·85	1·2	0·6	8·8	8·1
10	8·02	8·33	14·77	8·00	6·42	2·61	0·684	0·341	25·56	33·35	0·7	0·5	10·4	10·4
11	7·99	8·27	14·67	7·90	6·23	2·61	0·662	0·339	24·92	32·99	1·1	0·6	7·9	9·6
12	8·02	8·30	13·41	7·49	5·78	2·30	0·627	0·306	20·98	30·38	1·2	0·6	6·8	8·2
13	7·95	8·24	16·53	7·95	6·63	2·31	0·707	0·308	24·43	31·27	0·8	0·8	7·8	7·5
14	8·02	8·33	16·45	8·77	6·75	2·30	0·711	0·302	20·92	26·05	0·8	0·4	6·3	6·2
15	7·94	8·28	13·43	7·77	5·92	2·35	0·633	0·304	16·53	21·12	1·1	0·7	7·3	5·1
16	7·99	8·27	15·03	7·81	6·16	2·60	0·662	0·323	17·52	19·38	1·0	0·7	7·4	4·9
r	0·029	0·040	−0·234	0·007	−0·279	0·334	−0·335	0·238	−0·533*	−0·577*	0·086	0·262	−0·316	−0·425

For 14 D.F. 5 % point is 0·497; 1 % point is 0·623. (* Significant.)

coefficient of correlation with wireworms is, for the upper cores, $-0{\cdot}234$, and, for the lower cores, 0·007. Neither is significant.

(*c*) *Organic carbon.* The soil at Sheep's Green contained about twice as much organic carbon as that at Spinney Pasture. The mean amount in the upper soil was $6{\cdot}27 \pm 0{\cdot}47\%$, and in the lower soil $2{\cdot}56 \pm 0{\cdot}23\%$. The coefficients of correlation with wireworm infestation, $-0{\cdot}279$ and 0·334 for the upper and lower soils respectively, are non-significant.

(*d*) *Nitrogen.* The amount of nitrogen in the soil at Sheep's Green, like the organic carbon, was about double the amount present at Spinney Pasture at both levels. For the upper soil, the mean measurement was $0{\cdot}658 \pm 0{\cdot}042\%$; for the lower soil, $0{\cdot}329 \pm 0{\cdot}079\%$. The coefficient of correlation for the upper soil is $-0{\cdot}335$ and for the lower soil 0·238. Neither of these values is significant. It is interesting that these three measures of the organic material present in the soil appear to be uncorrelated with the incidence of wireworms at Sheep's Green, although they are significantly correlated at Spinney Pasture. The difference will be discussed below.

(*e*) *Calcium carbonate.* The soil at Sheep's Green contains a great deal of $CaCO_3$; the mean value for the upper soil being $21{\cdot}39 \pm 3{\cdot}50\%$ and for the lower soil $27{\cdot}23 \pm 4{\cdot}05\%$. It would appear that the wireworms tend to avoid the higher concentrations, for the coefficient of correlation between the number of wireworms at the sixteen stations and the $CaCO_3$ in the upper soil is $-0{\cdot}533$, and in the lower soil $-0{\cdot}577$. Both values exceed the 5% point of significance.

(*f*) *Available potash.* The mean measurement for the upper soil was $1{\cdot}1 \pm 0{\cdot}3$ parts and for the lower soil $0{\cdot}7 \pm 0{\cdot}2$ parts per ten thousand by weight. The coefficients of correlation with wireworm infestation, 0·086 for the upper and 0·262 for the lower soil, do not suggest any correlation between the two.

(*g*) *Available phosphoric acid.* The mean proportion present in the upper soil at the sixteen stations was $7{\cdot}8 \pm 1{\cdot}8$ parts per ten thousand, and in the lower soil $6{\cdot}6 \pm 1{\cdot}8$ parts. The coefficient of correlation between the number of wireworms and the measurements of P_2O_5 are $-0{\cdot}316$ for the upper soil and $-0{\cdot}425$ for the lower soil, neither of which is significant.

E. *Vegetation*

Our study of the vegetation at Sheep's Green was conducted on quite different lines from that at Spinney Pasture. Instead of making a quantitative estimate of the flora of each station, we set ourselves to find out whether there was a major qualitative difference in the plant cover of the easterly and westerly rows which might explain their very different wireworm populations. Having found such a difference, we extended our observations to see whether it held good for samples taken elsewhere in Sheep's Green and in other fields.

The principal plant species concerned were the grasses *Hordeum pratense*, *Dactylis glomerata* and *Agrostis stolonifera*; thistles, *Cnicus arvensis*, and buttercups, *Ranunculus acris*, *repens* and *bulbosus*. Other species, including *Phleum pratense*, *Lolium perenne*, *Festuca ovina*, *Poa* sp., *Trifolium repens* and *Crepis virens*, occurred so much less commonly that they can be left out of the following summary account.

To compare the flora of the two rows in some detail, we examined the vegetation of twelve quadrats, each 1 yd. square, six along the line of the easterly row and six along the westerly row. The number of thistle plants (many of them small) was counted, and the percentage area occupied by buttercups and by the different species of grasses was estimated. The result, summarized in Table 9, shows that while the principal grasses differ little between the two rows, both the thistles and the buttercups were more numerous in the westerly row. This is especially noticeable in the case of *Ranunculus*, which occupied 23% of the area of the quadrats in the westerly row but only 1% in the easterly row.

Table 9. *Comparison of the principal flora of the two rows of stations at Sheep's Green (means of six square-yard quadrats along each row)*

	No. of *Cnicus*	Percentage area occupied by			
		Ranunculus	*Hordeum*	*Dactylis*	*Agrostis*
Easterly row	10	1	36	25	12
Westerly row	18	23	32	16	16

This set of observations is supported by another. As each of our soil samples was removed, the presence or absence of thistles and buttercups in its near vicinity was noted. Thistles were marked present if they occurred within about a yard, buttercups if they were within a foot of the sample. This was done at each of our samplings except that of September 1941, so that we have twenty records for each station spread over a period of 3 years. The information is summarized in the first two columns of Table 10, where each entry represents the number of samples out of

Table 10. *The numbers of some plants and insects found at the sixteen stations at Sheep's Green*

Station	No. of		No. of			% of *obscurus-lineatus*
	Cnicus	*Ranunculus*	*Athous*	*Agriotes sputator*	*Agriotes obscurus-lineatus*	
1	4	3	42	27	1	3·6
2	8	4	17	99	0	0
3	5	2	12	114	0	0
4	7	3	28	113	3	2·6
5	13	0	10	63	0	0
6	4	1	22	78	0	0
7	1	4	13	155	0	0
8	5	3	5	67	4	5·6
9	5	6	2	8	12	60
10	12	16	15	21	10	32·3
11	10	17	5	6	16	72·7
12	11	17	9	12	30	71·4
13	11	13	0	8	18	69·2
14	13	6	1	8	12	60
15	10	1	2	15	13	46·4
16	10	0	17	63	5	7·4

twenty on or near which the weed occurred. It is evident at a glance that both thistles and buttercups were much more plentiful in the westerly than in the easterly row. On the average, there were 5·8 observations of *Cnicus* per station in the easterly row and 11 in the westerly. For *Ranunculus*, the difference was even more marked; the means being 2·9 observations per station in the easterly row and 10 per station in the westerly row.

This clear difference between the two rows led us to investigate a little further the apparent avoidance of these two weeds by wireworms with a view to distinguishing between them. Pairs of 4 in. samples were taken in such a manner that one sample was immediately adjacent to a weed, and included part of its rootstock, the other was 1 yd. away to the west, or, if that site was occupied by a weed, 1 yd. to the east. The wireworms found in the two samples were then compared.

For *Cnicus arvensis*, twenty-one pairs of samples were examined, five pairs at Sheep's Green, eight pairs at Spinney Pasture, and eight pairs at a field near Swavesey, Cambs. The twenty-one samples near the plants contained a total of 247 wireworms; those away from the plants contained 214. The differences between the twenty-one pairs are non-significant ($t = 1{\cdot}09$; $P > 5\%$). There appears to be no general correlation between the occurrence of thistle roots in a soil sample and the number of wireworms it contains.

For *Ranunculus* only eleven pairs of samples were examined, five concerning *R. acris* and six *R. repens*, all collected at Sheep's Green. The eleven samples near the plants contained a total of thirty-eight wireworms, those away from the plants contained seventy-three. Although these totals seem to indicate avoidance of *Ranunculus*, the *t*-test shows that the differences between the eleven pairs are non-significant ($t = 1{\cdot}77$; $P > 5\%$). We wish that we had done more pairs of samples involving *Ranunculus*. So far as they go, these samples do not support any hypothesis that the low wireworm infestation of the westerly row was due to the number of buttercups there, and the superficial correlation may be due to a positive connexion between soil moisture and *Ranunculus* similar to the negative correlation between soil moisture and wireworms.

F. *Fauna*

When we began regular sampling at Sheep's Green we were without assistance and were unable to spare time for the collection of the fauna other than wireworms. Only for the samples taken in 1944 have we faunal records comparable with those of Spinney Pasture, and they are too few to provide a safe basis for detailed correlations. It is worth recording, however, that the group of eight stations having a high wireworm infestation gave an average of 8·7 chilopods, 2·1 Coleoptera (mostly Staphylinidae), 38 ants and 0·9 Diptera (mostly Nematocera) per sample per station. The other eight stations, having a low wireworm infestation, gave 5·2 chilopods, 3·8 Coleoptera, 3·6 ants and 3·1 Diptera per sample per station in the 1944 samples. For what they are worth these group averages show positive correlations between wireworms and chilopods and ants, and negative correlations between wireworms and the other two, just as we found at Spinney Pasture. The positive correlation

between wireworms and ants is especially marked, not only in the actual figures available but also in the frequent mention of ants in the notes we made when collecting samples in which many wireworms were subsequently found.

The wireworm fauna of Sheep's Green includes species of *Adrastus* and *Athous* as well as *Agriotes*. Of *Adrastus* we found only sixteen individuals; but of *Athous* sp., probably *haemorrhoidalis*, we collected 547. Most of the *Athous* were obtained from square-foot and square-yard samples, but exactly 200 were found in samples taken at the sixteen stations, among which they were distributed as shown in Table 10. At most of the stations a correspondence can be seen between the number of *Athous* collected and the infestation by *Agriotes*, but the correlation is spoiled by station 1, which provided more *Athous* than any other. The coefficient of correlation for all stations is 0·344, which is non-significant; but when station 1 is left out of account, the remaining fifteen have a coefficient of correlation of 0·687, which is highly significant.

One of the most interesting things about the distribution of wireworms at Sheep's Green is the manner in which the different species of *Agriotes* occurred. From the samples taken at the sixteen stations we collected fifteen adults and 1008 larvae over 6·5 mm. long. One adult was an *obscurus*, the other fourteen were all *sputator*. Of the larvae, 843 were *sputator* and 123 were *obscurus* or *lineatus*, which we have not attempted to distinguish in the larval stage. The other forty-two larvae could not be identified with certainty because the critical parts were injured or missing. The distribution of the 857 identified *sputator* and the 124 *obscurus-lineatus* at the sixteen stations is shown in Table 10, together with the percentage of *obscurus-lineatus* among the identified individuals.

It is immediately apparent that the two groups of stations distinguished by their total wireworm infestation are markedly different in the proportion of *obscurus-lineatus* in their populations. Stations 2–8 and 16, having a high wireworm infestation, average only 2% of *obscurus-lineatus*; stations 9–15 and 1, having a low wireworm infestation, average 52%. The negative correlation between the numbers of *obscurus-lineatus* and of *sputator* collected at the sixteen stations is highly significant ($r = -0{\cdot}727$, with 14 D.F.). This unexpected result raises a number of problems which will be discussed below.

G. *Factors influencing the distribution at Sheep's Green*

In the foregoing pages, it has been shown that the number of wireworms at the different stations at Sheep's Green was correlated to a highly significant degree with the altitude, with the depth of loam overlying the clay, and with the amount of soil moisture present at 3 and at 9 in. depth. We have first to try to discover which of these factors, if any, really influences the wireworm distribution.

On the scale of our plot there can be no direct effect of altitude on wireworms, and to explain this correlation we must look for associated factors. They are easily found. As would be expected, the altitude of the stations was negatively correlated with the amount of soil moisture at 3 and at 9 in. depth ($r = -0{\cdot}839$ and $r = -0{\cdot}800$, respectively, with 14 D.F.). Altitude was also closely correlated with the depth of

loam ($r = 0{\cdot}929$), for the simple reason that the varying depth of loam lying on a clay table largely determined the surface contours of the plot. These facts allow us to neglect altitude, and to look for an effective factor in the depth of loam or the soil moisture.

The coefficient of correlation between the incidence of wireworms and depth of loam was $+0{\cdot}683$, that between wireworms and soil moisture at 3 in. depth was $-0{\cdot}755$, and that between wireworms and soil moisture at 9 in. depth was $-0{\cdot}628$. All of these are highly significant; but the three factors are so closely interrelated that each coefficient derives support from the others. The calculation of partial correlation coefficients allows a clear discrimination among them. After eliminating the effects, in each case, of the other two factors, the partial correlation coefficient between wireworms and depth of loam becomes only $+0{\cdot}022$, that between wireworms and soil moisture at 3 in. depth $-0{\cdot}476$, and that between wireworms and soil moisture at 9 in. depth $+0{\cdot}195$, the sign having changed. There is therefore no doubt that the most important factor of these three is the amount of soil moisture at 3 in. depth. This conclusion is supported by other calculations, including some in which the number of wireworms in the top cores only were correlated with the soil-moisture measurements at 3 in. depth, and those from the bottom cores only with the measurements at 9 in. depth. These latter calculations add the information that, in the bottom cores alone, depth of loam was more important than the soil-moisture measurements at 9 in.

Interest in the correlation between wireworms and soil moisture at Sheep's Green centres about the fact that the correlation is negative. So much laboratory work has shown the dependence of wireworms on high humidity (Subklew, 1934; Evans, 1944) and the tendency for wireworms to migrate from dry to moist soils (Langenbuch, 1932; Lees, 1943*a*) that one is accustomed to think of them as preferring very damp soils. These field data from Sheep's Green (supported by the data from Spinney Pasture, although there the correlation does not reach the conventional level of significance) show wireworms to be much less numerous in the damp parts of the field than in the drier parts. This may have been due, of course, to the intervention of some other quite different factor—perhaps the *Ranunculus* we shall mention below—but we should like to suggest that the aeration of damp soils should be brought into consideration. Dr Lees tells us that in his experiments on the reactions of *Agriotes* to soil moisture (Lees, 1943*b*), he used sand 65% saturated, because, when the moisture content was higher, wireworms were asphyxiated. The sand used in his experiments was saturated when water weighing 23% of the dry weight of sand was added. At Sheep's Green, the average soil-moisture content at 3 in. depth at stations 1 and 9–15 was 34·3% of the dry weight of the soil. The saturation points of the sand used by Dr Lees and the soil at Sheep's Green were doubtless very different. But the question remains whether wireworms living in soil of high moisture content, where, owing to a shallow soil overlying clay, their burrows are likely to be flooded, are liable to asphyxiation. The part played by aeration of the soil in the apparent effects of soil moisture on wireworms seems to us worthy of closer attention than it has yet received.

The correlation between calcium carbonate and wireworms is significant at the 5% level for both the upper and the lower cores. This factor was not correlated with the wireworm distribution at Spinney Pasture, but there the average $CaCO_3$ content of the soil was only a third as high as at Sheep's Green. This suggests that while wireworms are indifferent to chalk at lower concentrations, they may tend to avoid it when its concentration reaches between 20 and 30%.

In the plot at Sheep's Green no correlation was found between wireworms and organic carbon, nitrogen, or loss on ignition, although at Spinney Pasture there was in each case a highly significant negative correlation. At Sheep's Green each of these factors occurred at a much higher level than at Spinney Pasture—the percentage of organic carbon and of nitrogen was about twice as high. This affords evidence that wireworms do not avoid the organic matter in the soil as such. It bears out the suggestion made above (p. 16) that the correlation at Spinney Pasture was due to avoidance of the grass *Lolium* rather than of the organic content of the soil.

There was a large amount of *Ranunculus* on the westerly row of stations where there were few wireworms. Comparative samples taken elsewhere in Sheep's Green, however, failed to show a significant correlation in general between the occurrence of buttercups and of wireworms. Unfortunately, our method of surveying the vegetation at Sheep's Green, from row to row rather than from station to station, does not allow statistical comparison with the soil factors discussed above. We can only point out that the amount of *Ranunculus*, possibly in relation to the amount of soil moisture, is a factor worth further study.

The presence of *Agriotes obscurus* and/or *lineatus* at some of the stations at Sheep's Green, and the highly significant negative correlation between the occurrence of *obscurus-lineatus* and *sputator*, raises several important questions. Does *obscurus-lineatus* usually inhabit wetter soil with a higher proportion of weeds among the grass? Some such qualitative difference in the habitat of the species is clearly indicated by our data (Table 10). The absence or scarcity of *obscurus-lineatus* at some stations cannot be explained as an accident of colonization, for our samples were collected over a period of three years, ample time for migration or colonization across 5 or 10 yd. distance. When they occupy the same site, is *obscurus-lineatus* actually antagonistic to *sputator*? Has a mixed population, including *obscurus-lineatus*, a lower maximum density than a nearly pure population of *sputator*; that is, would there be more *Agriotes* in the westerly row if there were not so many *obscurus-lineatus*? If the answer to this last question is in the affirmative, it is possible that the distribution of gross numbers of *Agriotes* at Sheep's Green, all the data with which we have been occupied, may be partly dependent on the difference in the distribution of the two species.

So far, then, as we have been able to carry our observations on the small experimental plot at Sheep's Green, the factor most closely correlated with the infestation by *Agriotes* is the amount of soil moisture present at 3 in. depth. The prevalence of buttercup plants among the grass may also be important, but our evidence about this factor is less satisfactory. Much interest attaches to the occurrence of *Agriotes* '*obscurus-lineatus*' at several stations, and its numerical preponderance at some

stations where the total *Agriotes* population is very small; but the general significance of the observation cannot be ascertained until more is known of the interactions of populations of *obscurus-lineatus* and *sputator*.

MICRO-DISTRIBUTION

In order to obtain information about the micro-distribution of wireworms, we have examined four square-yard samples. Each of them included all the soil from an area 1 yd. square, removed to a depth of 6 in., in eighty-one separate parts, each 4 in. square. The careful removal of the eighty-one separate quadrats occupied several hours, and it is possible that there was some migration of wireworms across the boundaries of adjacent quadrats in the course of the work. But the effect of such movements on our results is probably negligible; in the first place because wireworms tend to move up and down rather than sideways, in the second place because any lateral movements that did occur would be so ill-directed as to be practically random, and in the third place because it is doubtful whether even the large larvae could move through the soil quickly enough to cross more than one boundary before the adjacent sample was taken.

The first square-yard sample to be described, Square Yard A, was removed from Spinney Pasture half-way along its north-eastern edge. From that sample we obtained 1100 wireworm larvae and sixteen adults. The larvae were distributed in the different quadrats as shown in Fig. 3*a*.

The question is, were the wireworms distributed at random, independently of one another, among the eighty-one quadrats? If they were, the numbers of wireworms in the different quadrats should follow a Poisson series. That hypothesis is readily contradicted ($\chi^2 = 29{\cdot}47$ with 8 D.F.). The larvae are not distributed independently and at random within the square yard.

But the question can be answered more conveniently by means of the 'coefficient of dispersion' suggested by Fisher and used by Blackman (1942, p. 352) for the analysis of the vegetation of pastures. The formula

$$\frac{\Sigma(x-\bar{x})^2}{\bar{x}(n-1)},$$

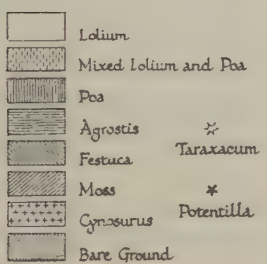
leads to unity when the individuals are distributed at random, to less than unity when they are over-dispersed (i.e. evenly distributed), and to more than unity when they are under-dispersed (i.e. aggregated). The significance of the divergence from unity can be tested by the formula $2\sqrt{\frac{2n}{(n-1)^2}}$, where n is the number of quadrats. For our sample of eighty-one quadrats, this amounts to 0·318, and a coefficient of dispersion that lies outside the limits $1 \pm 0{\cdot}318$ is significant of non-random distribution. In the case of Square Yard A, the coefficient of dispersion is 2·84, which is significant of aggregation and indicates that the wireworms tend to occur in groups.

This result can be tested against the other three square-yard samples we have examined.

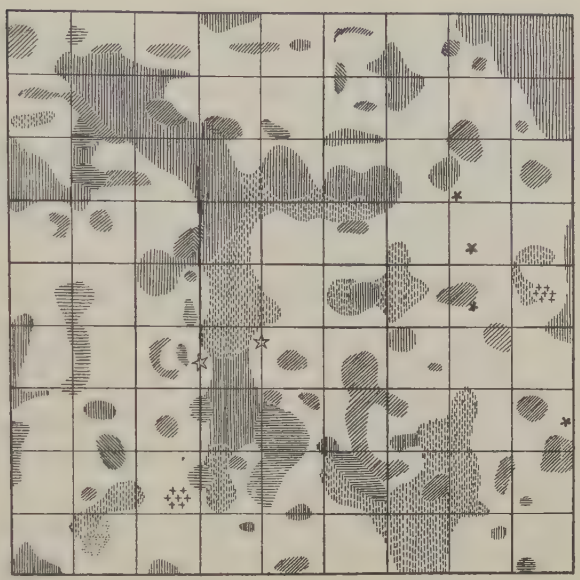
(1) Square Yard B was taken in the eastern corner of Spinney Pasture near station 19. From it were obtained 1334 larvae, one pupa and forty-five adults of

4	7	17	19	11	5	6	11	15
16	7	10	14	6	4	12	6	11
7	11	13	6	11	11	12	11	21
12	6	8	4	16	13	16	9	14
7	6	13	20	17	13	10	20	9
14	7	14	21	6	12	18	14	25
12	5	13	21	24	16	23	13	22
16	11	19	8	11	21	13	15	11
18	24	20	18	22	38	15	24	19

a



b



c

0	3	8	9	4	2	1	2	3
7	3	7	6	3	1	3	1	3
3	1	8	1	4	3	4	3	6
0	1	4	2	11	5	8	1	3
5	1	6	11	9	7	3	9	6
3	2	7	13	2	7	8	9	14
2	0	7	8	15	8	12	7	5
8	3	8	5	6	12	5	3	2
7	8	10	10	11	15	8	10	12

d

0	1	8	10	4	3	5	7	7
4	3	3	7	2	1	8	4	5
2	5	2	3	4	5	3	3	12
5	3	2	1	4	4	3	5	7
0	2	4	2	7	0	4	7	0
5	2	5	5	0	2	7	3	9
7	3	6	6	7	5	7	1	10
6	7	7	2	3	3	3	7	7
6	7	9	4	9	18	5	12	6

e

4	3	1	0	3	0	0	2	5
5	1	0	1	1	2	1	1	3
2	5	3	2	3	3	5	5	3
7	2	2	1	1	4	5	3	4
2	3	3	7	1	6	3	4	3
6	3	2	3	4	3	3	2	2
3	2	0	7	2	3	4	5	7
2	1	4	1	2	6	5	5	2
5	9	1	4	2	5	2	2	1

f

0	1	5	8	2	1	2	2	0
0	1	1	3	1	2	0	2	0
1	1	2	0	2	0	7	3	13
3	0	5	3	2	4	6	2	4
5	1	4	2	2	1	3	3	2
1	2	2	2	0	3	15	3	4
6	3	2	6	4	3	3	1	4
10	2	1	3	4	5	2	0	8
3	7	0	4	1	12	5	9	1

g

2	1	0	3	3	2	5	1	2
3	2	0	5	2	3	2	4	1
4	1	4	5	2	4	4	2	5
1	6	1	1	1	2	2	3	2
2	3	3	4	3	2	0	4	2
5	8	1	10	1	1	4	2	3
5	1	2	0	1	1	4	3	3
4	1	2	2	6	1	2	2	5
2	4	2	4	4	3	5	5	5

h

1	3	3	1	2	1	5	1	2
3	1	0	1	0	2	2	1	0
2	2	3	0	2	2	2	1	10
2	3	5	5	4	2	2	2	2
2	2	3	1	7	2	3	5	0
2	1	2	2	1	2	1	5	3
4	0	4	3	1	3	2	4	0
7	2	0	3	1	1	3	4	1
1	0	3	1	1	1	3	1	2

i

Fig. 3. Square Yard A. *a*, distribution of wireworm larvae in the upper quadrats. *b* and *c*, legend and map of vegetation. *d–i*, distribution of small, medium-sized, and large larvae, respectively, in the upper quadrats (*d*, *e*, *f*) and in the lower quadrats (*g*, *h*, *i*).

Agriotes. The distribution of the larvae among the eighty-one quadrats is recorded in Fig. 4*a*.

(2) Square Yard C was taken at Sheep's Green about 25 yd. south-east of station 9, and therefore well away from the experimental plot. It provided only 342 larvae and two adult *Agriotes* together with five *Athous*. The distribution of the larval *Agriotes* is shown in Fig. 4*b*.

(3) Square Yard D was taken at Sheep's Green, about midway between stations 5 and 15. We collected from it a total of 1139 larvae, sixteen pupae and two adult *Agriotes*, together with eighty-two individuals of *Athous*. The distribution of the *Agriotes* larvae is shown in Fig. 4*c*.

The coefficients of dispersion of the larvae in these three square yards are 3·33, 2·90 and 6·24 respectively. All are significantly different from unity and indicate that the larvae were not distributed independently and at random, and that in each case they were aggregated.

We can therefore draw the general conclusion that wireworm larvae in a grass field are not distributed at random, even within areas so small as 1 sq.yd., but tend to be aggregated into small groups.

Following the procedure adopted with the field and the plot, we should now go on to explore the environmental factors correlated with the distribution of wireworms within a square yard. When we attempt to do so, we are faced with an entirely different set of considerations. Within a square yard, many of the factors that we have hitherto investigated cannot be supposed to vary enough to influence the distribution of wireworms. Differences in topography are absurd, and differences in many properties of the soil are negligible, in the matter of inches that separates the eighty-one quadrats. Those factors can certainly be left out of account. Vegetation and the fauna are left.

Before taking each of the four square-yard samples, we carefully mapped the vegetation covering it. The map of Square Yard A is shown in Fig. 3*c*. But the vegetation observed on the surface of such a small area at any particular date bears little relation (1) to the root system beneath the surface, on which the wireworms feed; (2) to the vegetation of the area at other times of the year; and (3) to the vegetative cover that was present 1, 2, 3 or 4 years previously, when the different year groups of larvae were deposited and began to feed. We are therefore of the opinion that, although vegetative factors may be important in determining wireworm distribution, their importance cannot be revealed, though it may be illustrated, by the comparison of quadrats in square-yard samples.

Similar considerations apply to the fauna. Predators capable of destroying wireworms must be active enough to move easily through the soil of so small an area. Shall we expect them, at the particular moment of time when the sample was taken, to be numerous in a quadrat where wireworms are numerous, because they have moved there in search of prey; or shall we expect wireworms to be few where predators are numerous because they have been eaten? And surely the predators that ate the young wireworms in a quadrat 2 or 3 years ago have passed on, in more than one sense! At this stage of the analysis, we consider it useless to attempt a formal

26	16	7	15	24	25	14	37	15
13	13	12	29	34	22	6	14	9
19	9	16	21	20	15	7	8	5
10	16	22	5	12	8	12	9	10
10	13	17	14	19	22	26	24	26
7	9	6	6	24	19	18	22	22
10	21	12	7	15	19	15	11	15
25	15	32	14	23	28	24	22	32
13	24	17	15	4	12	14	20	16

a

6	4	7	6	1	4	6	4	5
4	6	7	1	0	2	3	3	3
7	5	4	4	0	7	4	1	1
11	7	8	6	5	1	2	1	6
4	0	4	15	18	2	0	2	2
5	1	4	8	4	7	2	0	3
4	3	5	2	5	6	1	2	2
1	4	5	7	2	3	2	2	1
0	1	6	6	6	2	18	9	4

b

23	13	3	15	10	8	8	9	6
5	9	11	10	11	11	8	10	7
11	6	12	21	8	6	8	22	4
54	24	4	11	20	20	20	16	14
10	6	11	12	20	6	24	54	19
15	11	11	12	19	12	16	20	28
11	16	6	40	17	6	8	13	36
12	16	10	14	11	11	14	18	7
25	13	17	11	17	4	7	9	6

c

22	12	4	7	12	20	9	32	11
9	9	5	23	17	13	6	10	3
7	8	10	14	17	7	5	1	5
5	5	12	3	9	5	3	5	5
3	2	5	7	7	15	18	14	10
2	2	4	4	18	15	12	12	20
9	16	5	5	8	17	8	6	4
20	12	19	9	19	18	15	12	20
8	15	11	11	2	10	9	11	12

d

2	1	0	0	0	0	5	3	3
0	0	0	0	0	0	2	3	2
0	1	0	1	0	1	0	0	1
0	1	2	3	1	0	0	1	1
1	0	0	8	9	0	0	0	0
0	0	0	2	2	6	2	0	0
0	1	0	0	5	3	0	0	0
0	0	1	3	0	1	0	1	0
0	0	1	2	2	1	16	4	0

e

10	7	0	5	1	0	0	0	0
1	0	1	5	4	1	0	0	1
8	1	4	3	2	1	1	6	0
48	15	0	1	1	4	3	5	0
4	0	1	0	0	0	7	30	11
5	0	1	1	3	3	3	13	19
0	1	0	26	9	1	0	4	32
2	7	0	3	1	0	1	8	1
14	5	6	7	13	1	0	2	0

f

1	3	2	4	8	4	2	4	1
4	0	4	3	12	6	0	3	2
6	0	5	2	2	8	2	4	0
4	9	7	2	1	2	3	3	4
3	7	7	4	12	6	5	5	10
3	2	1	1	4	3	5	7	1
0	4	7	2	6	2	4	2	6
2	1	6	3	1	8	6	8	10
3	5	2	1	0	2	2	7	3

g

3	3	6	3	0	1	1	0	1
4	4	5	1	0	0	1	0	0
6	3	3	2	0	4	3	1	0
7	5	3	3	3	1	0	0	1
2	0	4	7	8	2	0	0	1
4	1	3	4	1	1	0	0	1
4	2	5	2	0	2	0	2	0
1	4	4	2	1	2	2	1	1
0	1	4	4	4	0	1	3	3

h

8	3	2	6	2	2	0	1	2
1	3	5	4	3	2	5	3	2
2	1	2	5	2	2	5	9	3
2	3	3	5	12	6	10	5	5
3	4	5	8	10	4	10	17	3
4	9	2	8	11	6	10	7	2
8	10	3	8	4	2	5	5	1
8	7	5	5	7	6	3	2	3
6	5	9	2	1	0	2	5	5

i

3	1	1	4	4	1	3	1	3
0	4	3	3	5	3	0	1	4
6	1	1	5	1	0	0	3	0
1	2	3	0	2	1	6	1	1
4	4	5	3	0	1	3	5	6
2	5	1	1	2	1	1	3	1
1	1	0	0	1	0	3	3	5
3	2	7	2	3	2	3	2	2
2	4	4	3	2	0	3	2	1

j

1	0	1	3	1	3	0	1	1
0	2	2	0	0	2	0	0	+
1	1	1	1	0	2	1	0	0
4	1	3	0	1	0	2	0	4
1	0	0	0	1	0	0	2	1
1	0	1	2	1	0	0	0	2
0	0	0	0	0	1	1	0	2
0	0	0	2	1	0	0	0	0
0	0	1	0	0	1	1	2	1

k

5	3	1	4	7	6	8	8	4
3	6	5	1	4	8	3	7	4
1	4	6	13	4	3	2	7	1
4	6	1	5	7	10	7	6	9
3	2	5	4	10	2	7	7	5
6	2	8	3	5	3	3	0	7
3	5	3	6	4	3	3	4	3
2	2	5	6	3	5	10	8	3
5	3	2	2	3	3	5	2	1

l

Fig. 4. *a*, *b*, *c*, distribution of wireworm larvae in Square Yards B, C, D respectively. *d*–*l*, distribution of the small (*d*, *e*, *f*), medium-sized (*g*, *h*, *i*), and large larvae (*j*, *k*, *l*) in Square Yards B, C and D respectively.

correlation either between wireworms and vegetation or between wireworms and predatory animals, on the basis of these square-yard samples.

But the very considerations that seem to stifle the investigation in one direction, stimulate it in another. The difficulty of dealing with the vegetation and the fauna of small areas brings out in strong relief the dependence of present distribution on past history. It reminds us that to understand completely the present distribution of wireworms in a plot, we should have to be able to trace the movements of each larva and to discover the influences that brought it where it is. That we cannot do. But we can take a step towards it, and perhaps all that is needed for practical purposes, by considering the distribution, not simply of the total population of wireworms, but of the several age groups included in it; by having regard, that is, to the composition as well as the size of the wireworm population.

DISTRIBUTION AND POPULATION COMPOSITION

To this point in the account of our investigation we have been concerned exclusively with the observed distribution of wireworms. It is time now to turn our attention from the distribution to the processes that brought it about. Scrutiny of the problem suggests that within areas of the size of a field or smaller, three processes are involved. (1) *Oviposition*. Adult females, in four or five successive years, have selected the particular places in which they have laid their eggs. (2) *Movement*. The larvae hatching from those eggs were free to redistribute themselves, and some have moved about during 4 years, others during 2 or 3 years, but the youngest ones during only part of 1 year. (3) *Survival*. Many of the larvae originally hatched have died, and it cannot be assumed that the agents of death were regularly distributed.

These processes are obscured in any investigation that deals only with the total number of wireworms. But if data are available on the composition of the population, an attempt can be made to distinguish the separate effects of the three processes and to estimate their relative importance. For instance, the distribution of the recently hatched larvae, in August and September, must closely resemble the distribution of egg-laying, since these very small larvae cannot have moved far from where they hatched. Again, the distribution of the 4-year-old larvae must principally represent the effects of survival and movement, for those larvae have survived through 4 years and, during that time, have redistributed themselves in response to influences quite different from those that induced their parents to lay. For analysis of the processes of distribution, therefore, information is needed on the age as well as on the distribution of the wireworms.

Some, but not all, of the means for that analysis are now available. Miss J. F. Blacklocks (1944, and in unpublished work) has shown how larvae of *Agriotes sputator* can be separated into eight growth stages which represent, not absolute, but relative age groups. If all of our thousands of larvae had been assigned to those eight growth stages, we could proceed now to consider the distribution of each of the age groups they so nearly represent. Unfortunately, that laborious task is scarcely begun and is not likely to be completed in the near future. Nevertheless, a preliminary attack on the problem can be made. In an earlier paper (1944, p. 58) we

have remarked that the size groups of larval wireworms show peaks which may indicate age groups. On the average, the smaller larvae will be the younger, and the larger larvae the older. All of our larvae are measured. We can therefore use the measurements that are available in place of the age determinations that are not, and, in a rough and preliminary fashion, push our analysis of the spatial distribution of wireworms a little further.

The collections of Square Yard A provide the first example. We have divided the 1100 larvae into three size groups, arbitrarily, but with guidance from the shape of the population-size histogram. This gives a group of 463 small larvae nearly a year old, for the sample was collected in June, before the new generation of eggs had hatched. They were distributed as shown in Fig. 3 *d*. The 392 medium-sized larvae, 2 and 3 years old, and the 245 large larvae, 3 and 4 years old, were distributed as shown in Fig. 3 *e* and *f* respectively.

Table 11

A. *Coefficients of dispersion of the wireworm larvae in eighty-one quadrats*

Test of significance for eighty-one quadrats, $1 \pm 0{\cdot}318$.

Square Yard	Small larvae	Medium larvae	Large larvae
A	2·52*	2·01*	1·21
B	3·60*	2·01*	1·27
C	4·56*	1·78*	1·20
D	13·69*	2·11*	1·39*

B. *Coefficients of dispersion of the wireworm larvae in eighty-one quadrats when the components for 9 sq.ft. are removed*

Test of significance for 72 D.F., $1 \pm 0{\cdot}336$.

Square Yard	Small larvae	Medium larvae	Large larvae
A	1·82*	1·73*	1·23
B	3·25*	1·83*	1·20
C	4·01*	1·19	1·11
D	13·14*	1·49*	1·39*

(* Denotes a significant deviation from unity.)

The coefficient of dispersion of the small larvae is 2·52, which is highly significant of aggregation. That of the medium-sized larvae is 2·01, which is also significant of aggregation. But the large larvae have a coefficient of dispersion of only 1·21, which is not significantly different from a random distribution. The larvae, gathered together in groups when they are young, seem gradually to disperse and to reach a random distribution when they are old.

The other three square-yard samples give similar results. In each case, groups of small, medium-sized, and large larvae were separated arbitrarily, after reference to the size-frequency histogram. The distribution of the larvae comprising those groups is shown in Fig. 4 *d–l*, and the coefficients of dispersion for the twelve size groups of all four square-yard samples are set out for comparison in Table 11 A.

In every case, the small larvae show a coefficient of dispersion very much greater

than 1·318 and therefore highly significant of aggregation. The medium-sized larvae were also significantly aggregated, although the coefficients are lower than those of the small larvae. The large larvae, on the other hand, were distributed in three samples in a manner that did not significantly differ from random. In the fourth sample, Square Yard D, the coefficient of dispersion of the large larvae slightly exceeds the limit of probability of a random distribution, but it is likely that in this case we have set the lower limit of size too low, for the group includes 369 larvae out of a total collection of 1139. When only the 119 largest larvae are included in it, the coefficient of dispersion of this group falls to 1·06, which is indicative of a random distribution.

The coefficient of dispersion is not entirely satisfactory for the analysis of our square-yard samples. It serves for quadrats taken at random, and wastes the additional information to be obtained from the arrangement of our quadrats within the square yard. To meet this case, Prof. Fisher has suggested that we make an analysis of variance on the sums of squares between the eighty-one quadrats and remove the component for the 9 sq.ft. This discloses what share the larger grouping contributes to the total distribution. The coefficient of dispersion of the remainder can then be tested as having 72 degrees of freedom. The results of these calculations are set out in Table 11 B.

The new calculations do not materially alter the situation. In each square yard there is a decrease in the value of the coefficient from the small larvae, through the medium-sized to the large. The principal result of the further analysis lies in the fact that, in almost every case, the new coefficient is smaller than the old. This indicates that the square-foot blocks contribute a disproportionate share of the aggregation: that the clumps of larvae tend to occupy a greater space than a single 4×4 in. quadrat. In the case of the medium-sized larvae of Square Yard C, indeed, the aggregation within the 9 square feet is so great as to reduce the coefficient of the remainder to non-significance.

These analyses of four square-yard samples provide a view of two of the processes of wireworm distribution. It appears that, as a result of the habit of the adult females of laying a number of eggs more or less together in a selected place, the young wireworms are markedly aggregated. In the course of time the growing wireworms disperse and, in a pasture, gradually assume a random distribution.

Two further comments are to be made. First, the date of collection of the square-yard samples affects the degree of aggregation of the group of small larvae. Square Yards A and C were taken in June, before the current season's eggs had hatched. The small larvae of these collections had had nearly a year to disperse from the oviposition site. Square Yards B and D were collected in August. The smallest larvae in these samples had therefore had less than a month in which to disperse. To some extent these differences appear in Table 11; but our crude separation of the larvae into three size groups obscures what we expect will be much more evident when separation into age groups has been accomplished.

Secondly, the dispersion from oviposition clumps seems to end, under grass, in a random distribution of the older larvae. Where the feeding conditions are less

uniform than they are in the upper soil of a pasture, the old larvae may regroup themselves into aggregations which are not relics of the oviposition clumps but new aggregations formed in favourable feeding sites. This is marked in arable land. In June 1943, forty samples were taken in a field sown with peas; twenty on the rows and twenty in the interrows. The samples from the rows contained 151 wireworms, of which eighty-four were longer than 8 mm.; the interrow samples contained forty-seven wireworms of which twenty-one were longer than 8 mm. The row samples contained over three times as many wireworms as the interrow samples, but four times as many large wireworms.

A similar aggregation of large larvae at feeding sites may take place in pastures if especially palatable food occurs among otherwise uniform grass. That does not seem to be represented in our surface samples, but it does seem to have occurred in the one square-yard sample we carried to a lower level. Underneath Square Yard A, a second tier of quadrats was removed, from 6 to 12 in. deep. From these eighty-one quadrats, 661 larvae and one adult *Agriotes* were obtained, additional to those in the upper 6 in. These larvae were separated into three groups with the same limits of size as those of the upper tier, and their distribution is shown in Fig. 3 *g–i*. The coefficient of dispersion of the small larvae is 2·88, which is highly significant of aggregation; that of the medium-sized larvae is 1·17, which indicates a random distribution; that of the large larvae is 1·38, which is again significant of aggregation. This suggests that, in the lower soil, beneath the general tangle of roots near the surface, the larvae disperse earlier in search of food, and then tend to concentrate about certain deeper root systems that are more attractive than others.

Applying our analysis of the processes of distribution to conditions at Sheep's Green, we must first emphasize the marked and persistent differences in the wireworm populations found at stations only a few yards apart. Stations 1 and 2 were only 5 yd. apart; some samples, indeed, only 1 yd. apart. Yet the total collection from station 1 was sixty-three wireworms; from station 2, 206 (Table 6). The only sample from station 1 that contained more than six wireworms was the sample of April 1942, the one nearest to station 2. A similar difference is found between the adjacent stations 8 and 9. Between stations 15 and 16 the difference is even greater, fifty-three wireworms as against 337; and two of the three samples from station 15 that contained more than five wireworms were samples taken 2 yd. south of the station point and nearest to station 16. There can be no doubt that both adult beetles and large wireworms are capable of moving 5 yd., the distance that separates adjacent stations. Either, therefore, adults avoid laying eggs at stations 1, 9 and 15, and larvae do not move to them, or most of the eggs and larvae fail to survive at those stations. The latter explanation seems to us untenable because, if eggs and larvae were at any season numerous at those stations, and subsequently died, our monthly (sometimes half-monthly) samples should have revealed that fact; which they did not. We must therefore conclude that adults and larvae both tended to avoid certain parts of Sheep's Green as a matter of behaviour; the adults did not lay there, the wireworms did not move there to feed.

Since a crude separation of the wireworm larvae into three size groups has served

to show something of the processes of distribution on the scale of a square-yard sample, it would seem logical to make use of similar divisions in our collections of larvae from the plot and the field. For two reasons, however, that is not possible. First, for statistical correlation with measurements of various factors, accurate numbers of the wireworms concerned are essential. The arbitrary separation into size groups that we have found satisfactory for a qualitative analysis would not be sufficiently precise for quantitative work leading to the calculation of a correlation coefficient. Secondly, with a group of samples taken all at one time, such as the eighty-one quadrats of a square-yard sample, a single arbitrary selection of size limits suffices to separate the larvae into the three groups. But for a collection of samples taken in every month of the year, such as those from the field and the plot, different limits would have to be fixed for each monthly collection to allow for the growth of the larvae. The accumulated error from the fixing of twelve arbitrary limits would be so great as to give no confidence in the size of the groups so fixed.

For these two reasons we cannot now reopen the problems of field and plot distribution and consider them again, as we should like to do, with regard to population composition. To overcome this difficulty by separating the larvae into their true age groups, and then to evaluate the three processes of distribution on a field and plot scale, would be an important step forward in the problems of wireworm distribution. Correlations between the youngest larvae and the various environmental factors would lead directly to a knowledge of the field conditions that attract the adult beetles to lay. Those between the old larvae and other factors would reveal something of the movements and much more of the survival and natural destruction of wireworms. There is little hope that our thousands of larvae can be examined and put into their age groups in the near future. But when that can be done, we shall be able to return to these problems of spatial distribution in another paper.

A NOTE ON GEOGRAPHICAL DISTRIBUTION

The distribution of wireworms on a scale larger than that of a field lies outside the limits of this investigation. However, in the course of our work we have made two sets of observations bearing on geographical distribution, and it will be more convenient to mention them here than to put them under another title.

It was shown by the National Wireworm Survey of 1939–40 that the wireworm populations of grass fields decrease from the south and east of England to the north and west (Adv. Entom. Conf. 1944). In the northernmost counties of England, the species of *Agriotes* cease to be important agricultural pests; and in the southern half of Scotland they become increasingly uncommon. We can find no records of *sputator* or *lineatus* north of the Grampians, and only four of *obscurus*, three from Aviemore and one from Nethy Bridge, kindly communicated to us by Mr D. K. Kevan.

Consideration of the life history of wireworms and of what is known of their temperature relationships, together with the climate of the British Isles, led us to think it likely that the northern limit of *Agriotes* might be set by the minimum temperature required for pupation.

Briefly, the argument is this. Temperature could only be efficient at the northern

limit if it were lethal in winter to the larvae or adults, or if it were too low in summer for the egg or the pupal stage. In view of the low temperatures that wireworms are able to withstand (cf. Falconer, 1945), and considering that the average temperature of Cambridge and Aberdeen is the same in December and January and that the daily minimum temperature is actually lower at Cambridge than at Aberdeen from November to March (Bilham, 1938, pp. 316, 327), the first alternative can be put aside. As for the second, eggs are laid in the region of Manchester from 1 May (Miles & Cohen, 1941), long before the warmest time of the year. Egg-laying and hatching do not seem to require high temperatures. Pupation, however, takes place only when the soil is at its maximum temperature, and the pupal period occurs in late July and during August. In those two months, the daily maximum temperature of the air at Aberdeen is 9° F. lower than that of Cambridge; and the soil is warmed by sunshine at Cambridge for 6·1 hr. a day on the average, but at Aberdeen for only 4·7 hr. (Bilham, 1938).

With these considerations in mind, we set up a simple experiment, intended to be preliminary to further work. Early in June, 176 large *Agriotes* larvae, apparently full grown and ready to pupate, were isolated in rearing tubes; and forty-four (thirty-four *sputator*, ten *obscurus-lineatus*) were put into each of four constant-temperature rooms, at 10, 15, 20 and 25° C. It is unnecessary for our present purpose to describe the methods and results of the experiment in detail, and it will suffice to record that within the next 5 months, thirty larvae pupated at 25° C., twenty-one at 20° C., six at 15° C. and none at 10° C. Of the six that pupated at 15° C., three were *obscurus* and three were *sputator*.

More critical work remains to be done. So far as it goes, this preliminary experiment shows that a soil temperature somewhere between 10 and 15° C., and probably near 15° C., is required for pupation. It may well be that on account of the cooler summers and the shorter periods of sunshine in the northern half of Scotland, the soil there does not reach and maintain the necessary temperature.

The second matter has to do with the geographical distribution of particular species of wireworms. In the report of the National Wireworm Survey (Adv. Entom. Conf. 1944, p. 26) information is given of the relative abundance of *obscurus*, *lineatus* and *sputator* in the several Advisory Provinces. These data, based on the collection of adult beetles, show that *obscurus* is the dominant species to the north and west, and *lineatus* to the south and west, and that *sputator* reaches its highest proportion in the east and south-east of England.

This last is the region in which wireworm populations as a whole reach their highest numbers. So far as the data go, therefore, they indicate a correlation between a high proportion of *sputator* and high wireworm populations.

It will be noticed that this is the same correlation as that found at Sheep's Green. On a geographical scale, as well as on a $\frac{1}{4}$-acre plot, high populations of *Agriotes* occur where there are most *sputator*, lower populations where *obscurus* (or *obscurus-lineatus*) is dominant. This supports the suggestion made above (p. 31) that *sputator* reaches a higher maximum population than the other species, or than mixed populations.

The coincidence can be carried a step further. The parts of England where *sputator* reaches its highest proportion are the driest parts of the country. The stations at Sheep's Green where *sputator* formed the high wireworm populations were the driest stations. There appears, therefore, to be a general agreement between high wireworm populations, the prevalence of *sputator*, and low moisture, both on a geographical scale and within a $\frac{1}{4}$-acre plot. A comparative study of populations of *sputator* and of *obscurus-lineatus* in relation to soil moisture seems to be needed.

DISCUSSION

A paper on the spatial distribution of wireworms might be expected to deal with three principal questions. The first, 'What is the distribution?' requires a descriptive answer. The second, 'What caused that distribution?' calls for analysis. The third, 'How did the observed distribution come about?' requires again a descriptive answer, but this time the description not of a state but of a process.

The spatial distribution of wireworms has been described above as it was found in a field, a plot, and in four areas of 1 sq.yd. The descriptions provided are, of course, peculiar to those particular areas, and to the particular period or time of sampling, but on two matters they provide general information.

First, in each of our experimental areas, the wireworms were distributed in a non-random fashion. Within a grass field of 8 acres, certain parts were consistently more heavily infested than others; and during 28 months, one part had a wireworm population only one-eighth as large as that of the remainder of the field. Within a grass plot of $\frac{1}{4}$ acre, the distribution was strikingly non-random over a period of 3 years; some places being consistently three or four times as heavily infested as other places only 5 yd. away. Within each of four square-yard samples examined at a particular time, the wireworms were aggregated into groups to an extent far greater than can be attributed to chance. All this is not to say that wireworms in general are not distributed at random, although that may seem likely. What it does show is that upon none of these scales, neither within a field, nor within a square yard of it, can wireworms be assumed to be distributed at random.

Secondly, in our square-yard samples, the small larvae were markedly aggregated, the medium-sized larvae more dispersed, the large larvae distributed at random. Since fields are but mosaics of such small areas, the distribution of wireworms under grass can be described in general as forming a sort of pattern—a group of small larvae here, a less compact group of medium-sized larvae there, with the large larvae more generally distributed among them—a pattern which is repeated again and again over the field, differing in the number and spacing of its elements from place to place so as to be never quite the same although retaining an essential similarity. This is a zoological example of a phenomenon that botanists have already met and that A. S. Watt (1943) has recently described as a 'pattern'.

The distribution of wireworms in an area having been described and shown to be non-random, the second question arises, 'What caused that distribution?' An answer to this question involves analysis of the wireworm environment, and study of the many factors composing it. That is so large a subject that no single investiga-

tion could be expected to lead to a complete answer. In the present study we have measured a number of edaphic factors in each of two larger areas and have subjected the measurements to statistical analysis so as to show up the factors most likely to be important (summaries, pp. 16 and 29). But not until those factors, and others, have been studied on many other fields in different parts of the country will it be possible to define with any certainty the conditions that govern the distribution of wireworms.

Since some factors of the wireworm environment have been the subjects of detailed laboratory studies, we might be expected at this point to relate our findings to those studies. For several reasons that would be unprofitable. In the first place, few of the published data refer specifically to *Agriotes sputator*, the species with which we have been mainly concerned. Observations reported above (p. 31) show that it may be quite incorrect to assume *sputator* to be physiologically similar to other *Agriotes*, let alone to other unspecified 'wireworms'. Secondly, so far as the published data concern distribution, they deal principally with the limits of tolerance of wireworm larvae for certain factors. But some of those limits are scarcely ever met in nature in England and, in any case, the problem of wireworm distribution within fields or from field to field requires information, not of the limits between which wireworms can exist, but of the conditions between those limits that encourage or discourage the building up and maintenance of high populations. Thirdly, and most important, practically all the published data have to do with large wireworms. Little or nothing is known even about the limits of tolerance or the behaviour of the small larvae, or of their ability to move away from adverse conditions. And yet these small larvae comprise a half or three-quarters of a natural population. Finally, the conditions controlling the distribution of wireworm populations in nature are unlikely to depend upon any single factor, but rather upon a much more subtle combination of many factors. In view of these considerations, it is not surprising that the experimental work available, excellent as some of it is, does not go far towards answering the question under discussion.

The third and final question that we might be expected to deal with in discussing wireworm distribution is 'How was the observed distribution brought about?' To that question we have devoted a separate part of this paper (p. 36), where we have suggested that three distinct processes are involved—oviposition, larval movements and survival.

We have been able to demonstrate the first two of those processes in a preliminary fashion, as they affect the micro-distribution of wireworms. Further than that we cannot go at present. Throughout this investigation of the distribution of wireworms we have naturally been involved with some of the major problems and difficulties of ecology. This final question, about the processes of distribution, brings us squarely up against the greatest of them—the impossibility of understanding distribution without having regard to succession. This is a difficulty that pervades the whole subject, but our investigation of the micro-distribution of wireworms affords an example of classroom clarity. A group of eggs is laid and the larvae gradually disperse, like the ripples from a stone dropped into water. But when

the dispersion is only one-quarter completed, another group of eggs is laid, not usually in exactly the same place, and a second wave of dispersion begins. At any one time, at least four such dispersions of larvae are taking place, and the relic of a fifth can be found in the distribution of the pupae or of the adults in their pupal chambers. These waves of dispersion, naturally, are not independent; they interact, and further complicate the situation that the observer finds when, at a particular moment of time, he comes to describe the distribution. What he finds, expressed as a number of individuals distributed thus and thus in space, can have little meaning unless time is introduced into the description and the evolution of the observed distribution is traced.

In short, spatial distribution and seasonal distribution are inseparable, and any study of the one without a study of the other is bound to be incomplete. For that reason this paper on spatial distribution cannot be brought to its real conclusion until another paper, on the seasonal distribution and population composition of our collections, has been written.

SUMMARY

1. Twenty stations were established in a grass field of 8 acres, and twenty-nine soil samples were examined from each station in the course of $2\frac{1}{3}$ years. Wireworms (*Agriotes sputator*) were distributed among the twenty stations in a non-random manner.

2. In this field, the wireworm distribution was correlated to a highly significant degree with nine factors (loss on ignition, organic carbon, nitrogen, *Lolium*, *Agrostis*, Chilopoda, staphylinid larvae, ants and Nematocera) and to a significant degree with three factors (altitude, *p*H of the soil and Diplopoda).

3. Among these factors, three (the organic content of the soil as measured by loss on ignition, the prevalence of the grass *Lolium*, and the numbers of staphylinid larvae) were found by analysis to be most significantly correlated with the wireworm distribution.

4. Sixteen stations were set up in a grass plot of $\frac{1}{4}$ acre, and twenty-one soil samples were examined from each station during a period of 3 years. The distribution of wireworms at these stations was markedly non-random throughout the sampling period, some stations being consistently three or four times as heavily infested as others only 5 yd. away.

5. Within this plot the distribution of wireworms was correlated to a highly significant degree with altitude, depth of loam and amount of soil moisture at 3 and at 9 in. depth. It was significantly correlated with the lime content of the soil. *Ranunculus* was prevalent on an area of low wireworm infestation.

6. Among the factors studied in this plot, the amount of soil moisture at 3 in. depth was most important. It was negatively correlated with the distribution of wireworms.

7. Within the plot, stations having high wireworm populations were infested almost exclusively by *Agriotes sputator*; those having low wireworm populations were infested about equally by *A. sputator* and *A.* '*obscurus-lineatus*'.

8. Four square-yard samples were examined, each in eighty-one quadrats 4 in. square. Within each square yard, the wireworms were not distributed at random and independently, but were markedly aggregated.

9. Separation of the wireworms collected from these samples into three size groups showed that the non-random distribution in square-yard samples was largely due to the small larvae, which were strongly aggregated. The medium-sized larvae were less markedly aggregated. The distribution of the large larvae under grass was not significantly different from a random distribution.

10. To understand the factors controlling wireworm distribution it will be necessary to consider the distribution of the different larval age-groups.

11. Two suggestions are made towards an explanation of the geographical distribution of wireworms in England.

12. Study of the spatial distribution of wireworms raises some of the major problems of ecology and, in particular, illustrates the interdependence of spatial distribution and seasonal succession.

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STUDIES ON TAPEWORM PHYSIOLOGY

I. THE CULTIVATION OF *SCHISTOCEPHALUS SOLIDUS IN VITRO*

By J. D. SMYTH, *Department of Zoology, University of Leeds*

(*Received* 1 *March* 1946)

(With Plates 1–3 and Five Text-figures)

INTRODUCTION

The lack of knowledge concerning the metabolism and general physiology of cestodes is mainly due to technical difficulties presented by their *in vitro* cultivation. Up to the present, attempts to cultivate parasitic stages of helminths have been relatively unsuccessful and in cestodes it is doubtful whether normal development has ever been obtained.

One of the major difficulties has been the absence of accurate information regarding the nutritional requirements of tapeworms, and thus 'any attempts to feed cestodes *in vitro* cannot be otherwise than empirical' (Wardle, 1934). A great variety of media have been used by various workers, including physiological salines —either alone or with the addition of carbohydrates, nutritive broths, serum, tissue culture media, etc.

Bacterial contamination is, however, the most important controlling factor in this type of cultivation. Since adult cestodes with one exception (*Archigetes sieboldi*, parasitic in the body cavity of oligochaetes) are all parasites of the alimentary canal of vertebrates, this difficulty is one of paramount importance, as the mucus film surrounding the worms is invariably rich in microflora. Rinsing the cestodes in dilute solutions of various bactericidal substances have met with little success (Wardle, 1934). The sedimentation technique—used with success by Ferguson (1940) for obtaining sterile trematode cercariae—has produced better results, and Wardle & Green (1941) succeeded in maintaining undulant activity in *Hymenolepis fraterna* without bacterial clouding for 20 days in dilute Baker's medium. In general, however, experiments with adult cestodes have been most unsatisfactory.

Larval cestodes being frequently localized in aseptic host tissues have given much more promising results. It seems likely that if a technique for the aseptic cultivation of larval tapeworms *in vitro* could be devised, and the conditions for the growth and development of the larva to the strobilar stage established, the problem of obtaining adult cestodes in a sterile condition would be largely solved.

The pseudophyllidean cestode *Schistocephalus solidus* Müller has many advantages for experiments of this nature, since its plerocercoid larva occurs in the body cavity of the three-spined stickleback *Gasterosteus aculeatus*, where it lives under completely aseptic conditions.

The problems to be solved briefly are: (*a*) the development of a technique for the removal of the larvae from the body cavity of the fish without bacterial contamina-

tion; (*b*) the provision of a suitable medium allowing the larvae to survive long enough for physiological experiments to be carried out; (*c*) the production of an appropriate stimulus to convert the larvae into adult forms.

PREVIOUS WORK

Frisch (1734) kept the plerocercoid larvae of *Schistocephalus* alive for 2 days in river water, and Abildgaard (1793) stated that the same larvae could be kept alive for 8 days in fresh tap water.

With regard to other cestodes, both larval and adult, a number of references are available, but only the more successful will be mentioned here. The results of culture attempts are summarized in Table 1. For a more detailed account of some of the earlier work, reference may be made to the reviews of Wardle (1937*b*) and Hoeppli, Feng & Chu (1938). Among the earlier workers, the most successful results were those of Lönnberg (1892) who kept *Triaenophorus tricuspidatus* alive in a dilute pepsin-peptone solution for over a month. More recently, Dévé (1926) kept the larvae of *Echinococcus granulosus* from the lung and liver of sheep alive for 2 weeks in aseptic hydatid fluid plus untreated horse serum. Coutelen (1927), using the same larvae and a medium of hydatid fluid plus various nutritive media, reported a viability of 31 days, and later (1929) kept *Coenurus serialis*, from rabbits, alive for 20 days, using normal saline plus fresh serum. The results of both Dévé and Coutelen are of particular interest as a transformation of the scolices of the cysticercoids was obtained, with considerable increase in size and bladder formation. Stunkard (1932) observed an increase of 3–4 times in length in larval *Crepidobothrium lonnbergi* kept alive for 32 days in dextrose-saline plus broth; segmentation of the strobila also took place, but only sterile and abnormal proglottids developed. The longest recorded viability *in vitro* for any cestode—larval or adult—was that obtained by Mendelsohn (1935) who cultured the larvae of *Taenia taeniaeformis* from a rat's liver for 35 days in sterile saline plus chicken embryo extract and filtered horse serum.

Wardle (1932, 1937*a*), working with the plerocercoids of *Nybelinia surmenicola*, *Triaenophorus tricuspidatus* and *Diphyllobothrium latum* under oligoseptic conditions using sterilized media, showed that, although complete asepsis was never obtained, the viability with unchanged sterile salines was considerably higher than with unsterilized media changed frequently. Larval *Nybelinia* gave the best results as they remained viable in double-strength Locke's solution up to 19 days. Earlier results with solid media were not satisfactory (1934), but recently Wardle & Green (1941) have reported the cultivation of the plerocercoids of *Diphyllobothrium latum* on various nutrient agar media, and in one experiment, using agar plus hog serum, considerable growth was obtained, although the total viability was only 5 days.

Joyeux & Baer* (1942) have investigated the cultivation of the plerocercoids of *Ligula*, a cestode closely allied to *Schistocephalus*. Larvae were cultured in Petri

* Published in France during the German occupation and only received in this country after the work in the present paper was completed.

Table 1. *Summary of previous attempts to culture cestodes* in vitro *in liquid media*

Reference	Species	Stage	Medium	Maximum viability in days
Frisch (1734)	*Schistocephalus solidus*	Plerocercoid	River water	2
Fabricus (1780)	*Proteocephalus percae*	Adult	Sea water	14
Abildgaard (1793)	*Schistocephalus solidus*	Plerocercoid	Tap water	8
Knoch (1863)	Various bothriocephalids	Adult	Aqueous solution of egg albumen	8
Zschokke (1888)	Cestodes from selachian fishes	Adult	Sea water + host gut mucus	1
Lönnberg (1892)	*Triaenophorus tricuspidatus*	Adult	Acid pepsin-peptone saline	28
Tower (1900)	*Moniezia expansa*	Adult	Albumen + beef extract + glucose	5
Ortner-Schönbach (1913)	*Calliobothrium coronatum*	Adult	Saline + monosaccharides	7
Le Bas (1924)	*Diphyllobothrium latum*	Plerocercoid	Ringer-Locke	7
Dévé (1926)	*Echinococcus granulosus*	Cysticercus	Hydatid fluid + serum	12
Coutelen (1927)	*Echinococcus granulosus*	Cysticercus	Hydatid fluid + ascitic fluid	31
Coutelen (1929)	*Multiceps serialis*	Cysticercus	Saline + horse serum	20
Cook & Sharman (1930)	*Moniezia trigonophora*	Adult	*M*/10,000 NaOH in carbonate-free Ringer	12
Wardle (1932)	*Diphyllobothrium latum*	Plerocercoid	M/20 NaCl	3
Stunkard (1932)	*Crepidobothrium lonnbergi*	Plerocercoid	Dextrose-saline + Hottinger broth	32
Brand (1933)	*Moniezia expansa*	Adult	Ringer	0·5
Wardle (1934)	*Triaenophorus tricuspidatus*	Plerocercoid	*M*/5–*M*/10 NaCl	2
Wardle (1934)	*Bothriocephalus scorpi*	Adult	Ringer-Locke	18
Wardle (1934)	*Nybelinia surmenicola*	Plerocercoid	Double Ringer-Locke	18
Mendelsohn (1935)	*Taenia taeniaeformis*	Cysticercus	Saline + chick extract + horse serum	35
Wardle (1937*a*)	*Moniezia expansa*	Adult	Tyrode	3
Hoeppli *et al.* (1938)	*Taenia taeniaeformis*	Cysticercus	Various nutrient serum solutions	18
Hoeppli *et al.* (1938)	*Diphyllobothrium erinacei*	Plerocercoid	Various nutrient serum solutions	18
Wardle & Green (1941)	*Hymenolepis fraterna*	Adult	Tyrode + Baker's medium	20
Joyeux & Baer (1942)	*Ligula intestinalis*	Plerocercoid	Various nutrient serum solutions	12
Wilmoth (1945)	*Taenia taeniaeformis*	Cysticercus	Ringer + glucose	24

dishes at 38–40° C. in (*a*) saline, (*b*) saline plus ascitic fluid, (*c*) saline plus horse serum, (*d*) saline plus serum and ascitic fluid. In the last three media, larvae remained viable for a maximum period of 12 days, and seven out of the twenty-four larvae used became apparently sexually mature and underwent oviposition. Sections revealed that normal development had not taken place, since spermatogenesis had not occurred. The addition of a number of tissue extracts were tried out in an attempt to induce spermatogenesis, but with negative results.

Wilmoth (1945) used a very wide range of simple and complex media in an attempt to find a suitable culture medium for the cysticercus of *Taenia taeniaeformis*, but with little success. Survival of this larva was longer in simple than complex media, and maximum viability of 24 days was obtained in Ringer's solution plus a trace of glucose.

LIFE CYCLE OF *SCHISTOCEPHALUS SOLIDUS*

According to Joyeux & Baer (1936), the strobilar phase of *Schistocephalus* occurs most commonly in mergansers and domestic ducks, but it has also been reported from numerous other birds frequenting marine and fresh-water areas. The adult is remarkable in that it only spends a very short period of its life cycle in the gut of the bird host, with the result that this phase is rarely found.

Kiessling (1882) fed a number of infected sticklebacks to domestic ducks and found that all the contained *Schistocephalus* were passed out with the faeces within 3–4 days. Unlike most other cestodes, the posterior proglottids are not cast off when ripe, but the entire worm is shed. The eggs are ejected from the uterine pores and pass out with the droppings of the bird.

The organism has the usual pseudophyllidean type of life cycle. The eggs which reach water hatch out in about 3 weeks into typical ciliated hexacanth embryos of the bothriocephalid type (Schauinsland, 1885). The free-swimming embryos are eaten by *Cyclops* spp., in the body cavity of which they develop into procercoids—as many as 60 having been found in a single crustacean. Nybelin (1919) experimentally infected *C. serrulatus* and *C. bicuspidatus*, and Callot & Desportes (1934) likewise infected *C. viridis*.

When infected *Cyclops* are eaten by a stickleback, the contained procercoids migrate through the intestinal wall of the latter and enter the body cavity where they develop into plerocercoids. Although the stickleback is the normal host for the plerocercoid phase, it has also been reported from *Atherina mochon* and *Blennius vulgaris* (Forti, 1932). Birds become infected by eating fish containing plerocercoids.

MATERIAL

The fish used in the present experiments were collected with a hand net from a large pond at Hunslet, Yorkshire. Throughout the course of this work several hundreds of fish were examined. *Every* fish collected from this area was found to be infected although the number of larvae per fish varied greatly. 4–10 larvae was a common number recorded, although a heavy infection of up to 140 was occasionally found.

Even the smallest fish collected (about 2 cm. in length) invariably contained one or more of the larvae. Infected fish show a very characteristic swelling of the abdomen which produces unnatural swimming movements. The plerocercoids lie in the body cavity (Pl. 2, fig. 1) packed close along the sides of the gut and between the other viscera which invariably are much compressed. Considering the extent of the infection—in some cases up to 40% of the entire weight of the fish—it is surprising that the larvae do not have a more apparent effect on the fish which reach their normal size in spite of the parasites. The plerocercoids of the closely allied form *Ligula intestinalis* produce temporary castration in *Atherina mochon*, but there is no evidence that the same condition occurs with *Schistocephalus*.

TECHNIQUE FOR ASEPTIC CULTIVATION

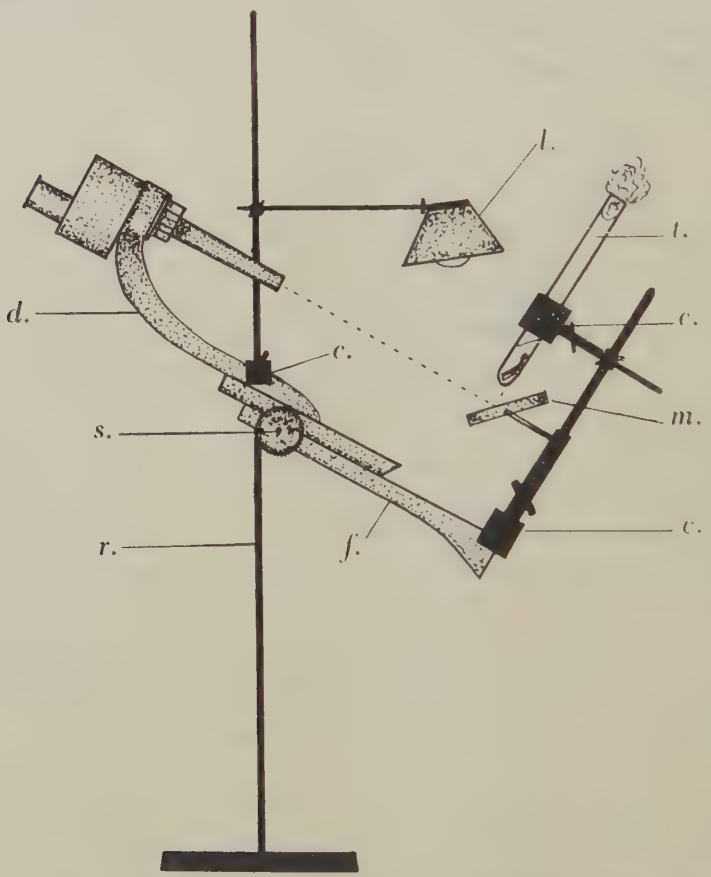
Infected fish were kept in the laboratory in fresh-running water under suitable conditions (Craig-Bennet, 1930) and killed as required by pithing. The technique for obtaining sterile larvae from the fish is somewhat elaborate, and some practice was necessary before aseptic cultures were obtained. The pithing was carried out by a long fine needle. The latter was pushed carefully and firmly down the neural canal so that a secure hold could be obtained on the fish for further manipulation without touching the skin. During the pithing, the fish was held by the skull only, as in heavy infections the larvae were forced out prematurely by the slightest pressure on the abdomen, rupturing the rectum and emerging through the anus with consequent faecal contamination.

Although the larvae lie in aseptic surroundings in the body cavity, it is further essential to sterilize the surface of the skin which is rich in microflora and with which the larvae may come in contact during the process of extraction. The skin was carefully dried with a soft cloth, and its entire surface painted with two coats of a saturated solution of iodine in absolute alcohol. The handle of the needle embedded in the fish was next secured in a bench vice, so that the fish was in a horizontal position with the ventral side towards the observer. The hands of the latter were thus freed for dissection. The upper part of the body cavity was slit open carefully with a sterilized cornea knife and the larvae, pressing closely against the body wall, immediately emerged to the outside; they were manipulated into the mouth of the tube containing the medium under investigation by means of a flamed platinum loop. It is important only to remove larvae from the anterior part of the body cavity, as worms in the posterior region may easily become infected by faecal droppings which sometimes exude through the anus. Care must also be taken to avoid puncturing the alimentary canal with the platinum loop during the manipulation.

The cultivation was carried out in plugged $15 \times 1{\cdot}5$ cm. test-tubes kept away from direct light at laboratory temperatures. Petri dishes were found to be unsuitable for cultivation as they became easily infected. As Wardle (1934) found Locke's solution (NaCl 9·0 g.; KCl 0·42 g.; CaCl 0·24 g.; $NaHCO_3$ 0·2 g.; distilled water 1000 c.c.) the most satisfactory saline medium for plerocercoids, various dilutions of this

medium—hereafter referred to as Locke—were used both pure and with the addition of glucose. The dilution is indicated by a prefix; thus $\frac{3}{2}$ Locke represents $1\frac{1}{2}$ times normal Locke's solution. The complete list of media used is shown in Table 2. As a nutrient medium, peptone broth was chosen; it was prepared from ox hearts following the usual procedure for bacteriological cultures (Bigger, 1935).

The medium in each tube was changed every 30 days, the renewal being carried out by means of a sterilized pipette. Owing to the refractive effects of the tube



Text-fig. 1. Arrangement of dissecting microscope for viewing larvae during cultivation *in vitro*. *c.* clamp; *d.* fixed column; *f.* sliding column; *l.* lamp; *m.* mirror; *r.* retort stand; *s.* coarse adjustment wheel; *t.* culture tube containing larva.

curvature, larvae could not be observed directly with a dissecting microscope. By partly dismantling and rearranging the microscope, however, it was possible to overcome this difficulty by viewing the reflection of the larvae from below (Text-fig. 1).

During the first day of cultivation, the larvae were examined for viability every hour; subsequently they were examined every 24 hr. Plerocercoids which were viable showed very marked undulant activity when subjected to a strong light for a few minutes—possibly a result of radiant heat effects rather than light. If no

undulation was observed, the viability was further tested by warming the tube by holding it in the vicinity of a small Bunsen flame for a few seconds; if alive, the larvae immediately responded to the stimulus. Larvae which failed to respond to this latter stimulus were considered dead.

The percentage of aseptic cultures obtained by this method varied from 75 to 100%. Cultures accidentally infected were easily recognizable as they became foetid and clouded within 2–6 days. In order to ascertain whether any histological or anatomical change had taken place during cultivation in the various media, larvae were fixed at intervals. Bouin was used for routine histological work and Carnoy and Champy for more detailed cytological study. Sections were stained in Heidenhain's iron-alum haematoxylin or Delafield's haematoxylin and eosin, the former giving much superior results.

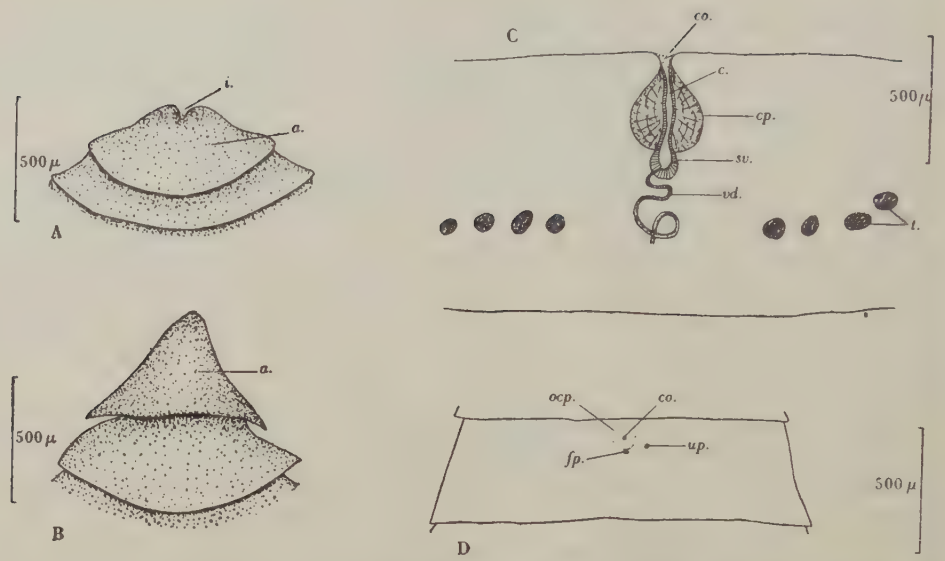
This preliminary series of experiments aimed at establishing a satisfactory aseptic technique as well as giving some indication of a suitable culture medium. When this was completed, a second series of experiments was carried out using the more favourable media incubated at the mean body temperature of the duck, 40° C. (Wetmore, 1921), in an endeavour to induce further development as in the bird host.

ANATOMY

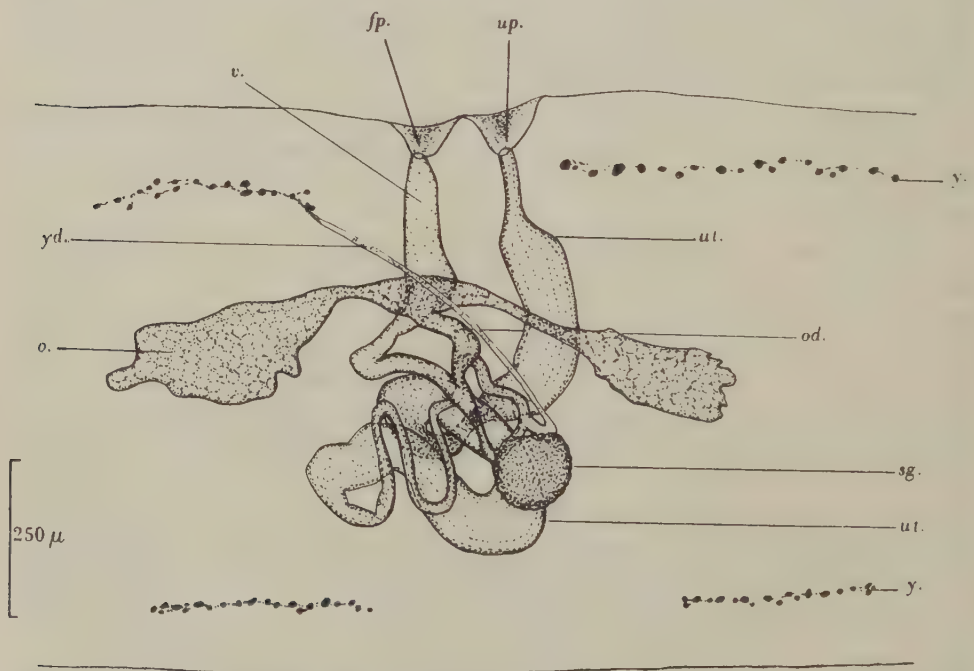
Schistocephalus solidus Müller, 1776 is the only species of the genus *Schistocephalus* Creplin, 1829, belonging to the subfamily Ligulinae Lühe, 1879, of the family Diphyllobothriidae Lühe, 1910. This species is unique among cestodes in that the plerocercoid phase is completely segmented.

The anatomy of the plerocercoid was studied from whole-mount preparations and from reconstructions from serial sections. Except for minor points, it agrees in general with that described by several authors (Moniez, 1881; Kiessling, 1882; Linton, 1927).

A typical well-developed plerocercoid (Pl. 1, fig. 1) consists of an anterior bothrial segment (*b*) followed by some 60–80 proglottids. In the larva as removed from the fish, the bothrial segment is bluntly rounded with a small anterior pit (Text-fig. 2A). During artificial cultivation this bothrial segment becomes everted and assumes the typical sharply-pointed adult condition (Text-fig. 2B). This eversion usually also occurs in whole-mount preparations (Pl. 1, fig. 1) on account of compression during fixation which is carried out between glass plates. The size of the larvae varied from 0·1 to 5·0 cm. (extended length)—the most frequently occurring length being between 2 and 3 cm. The genitalia reach a high grade of development in the larvae. A few clusters of testes cells appear in the 9th proglottid and the rudiments of the cirris can be seen in the 11th proglottid. From the 12th proglottid backwards, in forms larger than about 2 cm., the remainder of the genitalia are present in every segment. In some larvae, the genitalia in the most posterior three or four segments (Pl. 1, fig. 1, *a*.) are imperfectly developed, and although the rudiments of the cirris and the uterus are present, testes and yolk glands are either absent altogether or represented by a few cells only.



Text-fig. 2. A. Bothrial end of larva. B. Bothrial end of adult. C. Male genitalia of larva as seen in transverse section, other details omitted. D. Arrangement of genital openings in a proglottid. *a.* bothrial segment; *c.* cirris; *co.* cirris opening; *cp.* cirris pouch; *fp.* vaginal pore; *i.* anterior pit; *ocp.* outline of cirris pouch; *sv.* seminal vesicle; *t.* testes; *up.* uterine pore; *vd.* vas deferens.



Text-fig. 3. Female genitalia of the plerocercoid of *Schistocephalus*; reconstruction from serial sections. *fp.* vaginal pore; *o.* ovary; *od.* oviduct; *sg.* shell gland; *up.* uterine pore; *ut.* uterus; *v.* vagina; *y.* yolk glands; *yd.* yolk duct.

Histological examination reveals that the apparent advanced development of the genitalia is in fact superficial, with the cells of the reproductive organs in an immature condition. The ovaries (Pl. 2, fig. 4, *o.*; Text-fig. 3), lying in the lateral fields of each proglottid, consist of a compact mass of oval cells with large nuclei and little cytoplasm. A few cells are sometimes seen undergoing mitosis, but the number of actively dividing cells is small; meiosis has never been observed in the larval ovary. The oviduct is fully developed. The yolk glands are seen in sections as small unconnected clusters of round or spindle-shaped cells spread around the periphery of each proglottid except in the region of the genital pores (Pl. 2, figs. 2, 4, *yg.*). Each cell contains a large nucleus with a heavily staining nucleolus (Pl. 3, fig. 1; Text-fig. 5A); the cytoplasm is lightly staining and free from the typical granular appearance associated with yolk formation.

The testes which lie in the parenchyma on the side opposite the genital pores are enclosed in thin-walled membranes. Their condition varies somewhat in different larvae. For the most part they consist of a mass of oval or spindle-shaped cells which sometimes fill the entire capsule (Pl. 3, fig. 4) but which show no signs of differentiation. The number of cells, however, varies quite considerably, and in some testes there is a well-defined cavity. Mitosis is comparatively rare but has been seen in some specimens. The condition of the larval testes is therefore very primitive, and although the variable number of cells indicates that the cells can increase in number, no spermatogenesis takes place.

The cirris and seminal vesicle are fully developed, but the vas deferens, contrary to the description of Kiessling (1882), is single and shows no connexion with the testes at this stage but tails off into the parenchyma (Text-fig. 2C; Pl. 2, fig. 2). Even in the best preparations under oil immersion, no vasa efferentia could be located.

Since both medium-sized larvae of about 60 segments (approximately 2 cm.) and very large larvae of 80 segments (approximately 5 cm.) show genitalia in the condition described above, it is evident that the larvae can grow in size and increase the number of proglottids during their stay in the fish, but seemingly the genitalia cannot undergo any degree of differentiation or maturation until taken into the gut of the final bird host. As will be shown later, providing the food requirements are fulfilled, the stimulus for maturation of the genitalia is provided by the change in temperature between the fish and bird hosts.

CULTIVATION AT ROOM TEMPERATURES (16–19° C.)

(1) *General results*

The number of larvae surviving at intervals during cultivation at room temperatures in the various media are shown in Table 2. It must be emphasized, however, that the important factor to be measured is the length of time the larvae behave *normally* rather than the *total* viability *in vitro*. With the exception of the very toxic hypertonic media, two main abnormal features appeared after prolonged cultivation in all the media: cuticular peeling, and degeneration.

Cuticular peeling commenced by a loosening of the outer layer of the cuticle

(Pl. 1, fig. 3) at the posterior end of the organism and in extreme cases spread over the entire surface of the worm until the cuticle was completely stripped off. The extent of the cuticular peeling varied greatly, and in the most favourable media was barely noticeable and appeared only after very prolonged cultivation.

Degeneration commenced at the posterior end and gradually extended forwards. The tissue of the abothrial proglottids became flaccid and sometimes even transparent, although at the same time the anterior part of the organism remained active and exhibited marked undulation for some considerable time.

The appearance of these two features was used as a criterion for judging the period of normal behaviour. It is difficult, however, to decide the exact moment of transformation from normal to abnormal behaviour, so that the figures for the duration of normal behaviour *in vitro* given in Table 3 can only be considered accurate to within 2 or 3 days.

Table 2. *Viability of plerocercoids* in vitro *at room temperature*

Medium	No. of larvae used	No. of larvae surviving (cultivation time in days)				% of larvae surviving more than 140 days
		(20)	(60)	(100)	(140)	
2 Locke	23	0	—	—	—	0
$\frac{3}{2}$ Locke	10	0	—	—	—	0
$\frac{5}{4}$ Locke	15	0	—	—	—	0
Normal Locke	15	15	12	7	7	47
Normal Locke + 1 % glucose	11	8	6	6	4	36
Normal Locke + 0·5 % glucose	14	7	0	—	—	0
Normal Locke + 0·25 % glucose	10	7	0	—	—	0
$\frac{3}{4}$ Locke	10	10	10	10	9	90
$\frac{3}{4}$ Locke + 1 % glucose	16	12	0	—	—	0
$\frac{3}{4}$ Locke + 0·5 % glucose	28	19	2	2	2	7
$\frac{3}{4}$ Locke + 0·25 % glucose	23	21	4	4	1	4
$\frac{1}{2}$ Locke	23	19	14	12	7	30
$\frac{1}{2}$ Locke + 1 % glucose	21	1	0	—	—	0
$\frac{1}{2}$ Locke + 0·5 % glucose	42	1	0	—	—	0
$\frac{1}{2}$ Locke + 0·25 % glucose	25	9	4	2	1	4
Peptone broth	20	20	17	7	7	35

Nutrient peptone broth gave the most satisfactory results of all the media used, in that it gave the highest period of viability and normal behaviour, although the percentage of larvae surviving more than 140 days was less than in some of the salines. The activity in this medium was very marked, and cuticular peeling and degeneration, when they did occur, were slight. Of the twenty larvae used, seven were normal and active after 140 days' cultivation and one is still alive and exhibiting normal behaviour after 300 days' cultivation.

Of the various saline media tested, $\frac{3}{4}$ Locke gave the most satisfactory results with a mean period of normal behaviour of 114 days; nine out of the ten larvae used surviving for more than 140 days.

It is of interest to compare the behaviour of the larvae in the series of salines tested:

All larvae in 2 Locke, $\frac{3}{2}$ Locke and $\frac{5}{4}$ Locke died within 4 hr. During the first few minutes of immersion they showed only slight activity, followed rapidly by tetanic

contraction accompanied by a twisting and contortion of the organism and very marked cuticular peeling. There was no degeneration.

In normal Locke, larvae showed some contraction on first immersion, but regained their normal appearance after several hours. Cuticular peeling commenced in some larvae after 24 hr. but only to a very slight degree. The majority of larvae remained active though somewhat sluggish for 60 days; seven larvae survived for more than 140 days, but the maximum period of normal behaviour was 114 days. The addition of 1% glucose to the medium gave a higher maximum period of normal behaviour, but the mean was lower. The behaviour in normal Locke plus 0·5 or 0·25% glucose was very unsatisfactory, all the larvae dying within 50 days.

In ¾ Locke, the larvae behaved in a very normal manner, and even after 100 days' cultivation all the organisms reacted violently to light and in fact appeared little different from larvae freshly removed from the fish. Very marked degeneration, however, began after 106–131 days.

Table 3. *Duration of normal behaviour of plerocercoids during cultivation* in vitro *at room temperature*

Medium	Δ° C.	Period of normal behaviour in days			Standard deviation
		Minimum	Maximum	Mean	
2 Locke	−1·18	All dead within 30 min.			—
⅔ Locke	−0·88	All dead within 4 hr.			—
⅝ Locke	−0·74				
Normal Locke	−0·56	1	114	69	42·0
Normal Locke + 1% glucose	−0·66	1	130	54	44·2
Normal Locke + 0·5% glucose	−0·61	8	28	14	7·3
Normal Locke + 0·25% glucose	−0·59	3	22	12	6·5
¾ Locke	−0·42	106	131	114	8·9
¾ Locke + 1% glucose	−0·52	22	62	43	13·1
¾ Locke + 0·5% glucose	−0·47	8	122	25	28·6
¾ Locke + 0·25% glucose	−0·45	13	110	31	29·7
½ Locke	−0·28	7	131	45	62·3
½ Locke + 1% glucose	−0·38	8	17	12	4·0
½ Locke + 0·5% glucose	−0·33	8	18	10	5·8
½ Locke + 0·25% glucose	−0·31	3	112	30	36·1
Peptone broth	−0·56	34	300*	—	—

* One larva still very active and showing normal behaviour after 300 days' cultivation.

In ¾ Locke + 1% glucose, there was normal behaviour for the first 22 days, after which the majority of larvae soon became sluggish and all died within 70 days. In ¾ Locke + 0·5 or 0·25% glucose, the activity in the early stages was not so marked as in pure ¾ Locke, and cuticular peeling in general was apparent after only 8–13 days, though a small proportion of larvae remained viable and normal for over 100 days.

Some 30% of the larvae lived for over 140 days in ½ Locke, but the activity was not so marked and cuticular peeling commenced in some cases within 7 days. The hypotonicity of the medium produced considerable relaxation of the musculature, and the larvae became extended to about twice the length in the fresh condition. Behaviour in ½ Locke + 1 or 0·5% glucose was unsatisfactory, as about 50% of the plerocercoids died after 10 days; only one of the sixty-three used survived for 23 days.

The addition of 0·25% glucose gave somewhat better results, and although some larvae died within 8 days, four survived for more than 90 days and one for over 140 days. The general activity throughout, however, was sluggish, with the result that the normal behaviour time was low.

An interpretation of these results is difficult in view of the inconsistencies in the viability of the larvae in any one medium. For example, nineteen out of the twenty-eight larvae cultured in $\frac{3}{4}$ Locke + 0·25% glucose died after 20 days' cultivation, yet four lived for over 100 days, and one for over 140 days. It is probable that there are marked inherent differences between individual larvae or that there are many as yet unknown factors playing a part in the control of viability. The range of viability does, however, compare with that obtained by Brand & Simpson (1942) in cultivation attempts with nematodes.

Table 4. *Change in weight of plerocercoids after periods of immersion in concentrations of* NaCl

% NaCl	Δ NaCl (° C.)	No. of larvae used	% change in weight								
			30 min.			60 min.			90 min.		
			Series A	Series B	Mean	Series A	Series B	Mean	Series A	Series B	Mean
0·5	−0·298	20	+14·3	+11·3	+12·8	+19·4	+19·8	+19·6	+19·2	+18·2	+18·7
0·6	−0·357	19	+ 9·1	+11·1	+10·1	+13·5	+11·0	+12·3	+10·2	+12·4	+11·3
0·7	−0·409	18	+ 1·6	+ 3·0	+ 2·3	+ 0·8	+ 2·8	+ 1·8	+ 0·2	+ 0·7	+ 0·5
0·75	−0·444	18	+ 1·8	+ 1·5	+ 1·7	+ 0·4	0·0	+ 0·2	− 0·7	− 1·8	− 1·3
0·8	−0·472	11	− 0·8	− 1·7	− 1·3	− 3·8	− 3·8	− 3·8	− 5·3	− 3·8	− 4·6
0·9	−0·530	15	− 3·7	− 2·1	− 2·9	− 6·1	− 4·2	− 5·2	− 7·7	− 5·5	− 6·6
1·0	−0·588	15	− 8·6	− 8·8	− 8·7	−10·2	−10·0	−10·1	−11·8	−12·3	−12·1

(2) *Osmotic pressure of* Schistocephalus

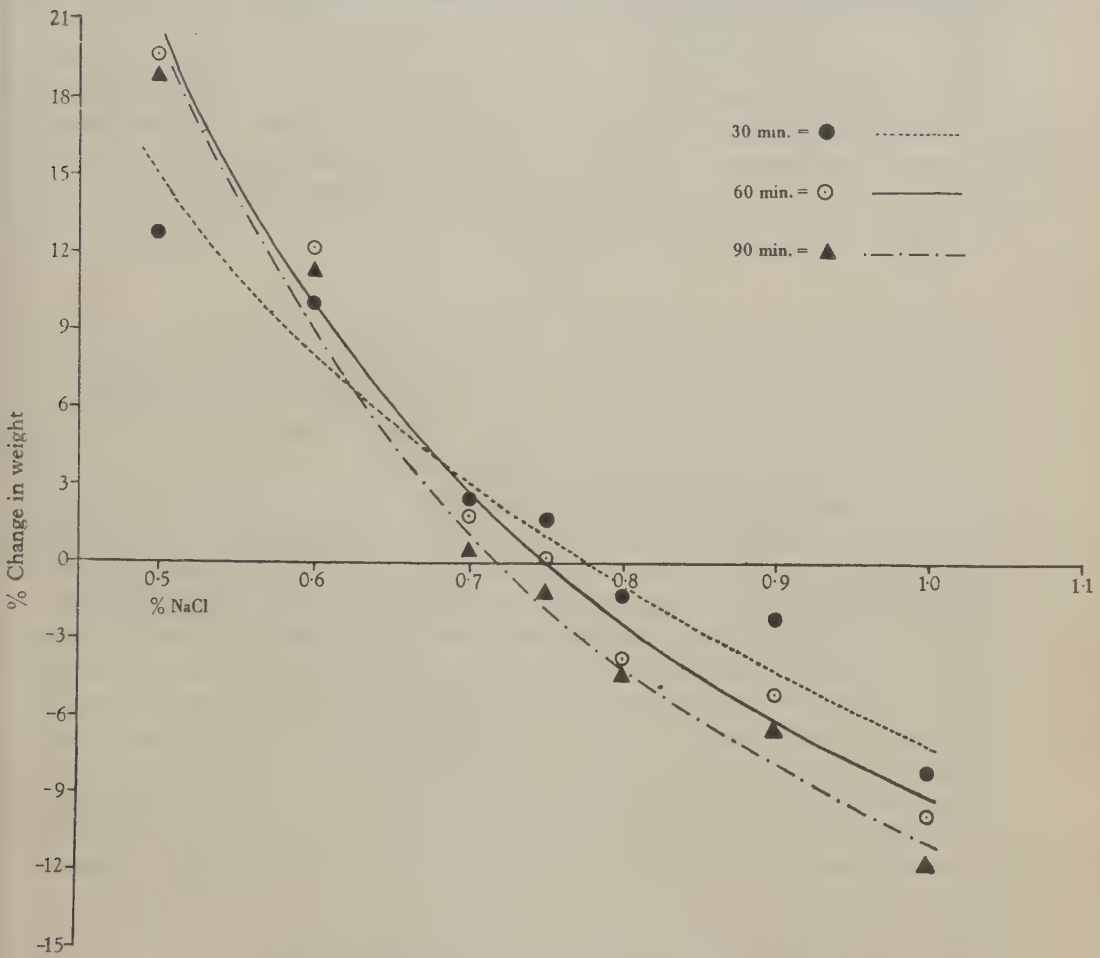
The osmotic relationships of the organism to the medium is a matter of some importance, and a subsidiary series of experiments was therefore carried out in order to obtain some knowledge on this point. The determination of the exact osmotic pressure of helminths in general presents a difficult problem. Schopfer (1932) prepared 'tissue extracts' of various cestodes and nematodes and with elaborate apparatus estimated the osmotic pressure cryoscopically. Estimation of volume change is a simple method easily applied to forms such as plerocercoids. The method involves the measurement of the change in weight after immersion in saline solutions of varying osmotic pressure. The main difficulty is to obtain 'reproducible removal of adhering water' (Krogh, 1939), but with care an accuracy of 2–3% can be obtained with this method.

After careful drying on filter paper, a number of larvae were weighed and immersed in solutions of 0·5, 0·6, 0·7, 0·75, 0·8, 0·9 and 1·0% 'Analar' sodium chloride in specially cleaned Petri dishes. They were removed, dried and weighed at intervals of 30 min. The results from two series of experiments are given in Table 4 and expressed graphically in Text-fig. 4.

It is only possible to base estimations on short periods of immersion, as the

behaviour becomes abnormal after 2–3 hr. in the higher and lower saline concentrations as already noted. It may be assumed that the change in volume is proportional to the change in weight, as density and metabolic changes are small enough to be neglected.

From the curves it is evident that the zero change in weight occurs in concentrations of sodium chloride approximately between 0·7 and 0·8%. The actual readings



Text-fig. 4. Graph of change in weight of plerocercoids of *Schistocephalus* after 30, 60 and 90 min. immersion in different concentrations of sodium chloride.

from the curves were: 0·72 (90 min.), 0·75 (60 min.), 0·78 (30 min.), with a mean of $0{\cdot}75 \pm 0{\cdot}3\%$. It is convenient to express the osmotic pressure of solutions in terms of the depression of the freezing-point (Δ). Taking into consideration the correction factor for the dissociation of sodium chloride at this concentration (*International Critical Tables*, 4, 1929), the value for the osmotic pressure of *Schistocephalus* is $\Delta = -0{\cdot}44 \pm 0{\cdot}02°$ C.

(3) *Histological examination*

Histological examination of the larvae cultured in any of the media for over 100 days at room temperature revealed that no further development had taken place, the condition of the genitalia being precisely the same as that of fresh larvae as removed from the fish. Throughout the entire cultivation period, no evidence of growth was recorded. The worms were measured as accurately as was possible through the walls of the culture tube and again after prolonged cultivation; the marked undulation made exact measurement difficult. It was not possible to weigh the larvae, owing to the technical limitations imposed by the maintenance of aseptic conditions. Nor was it practicable to count the number of proglottids of larvae within the tubes. It is possible, of course, that the degree of growth was so small as not to be measurable within the limits of the somewhat large experimental error involved, but further investigation is needed to decide this point.

CULTIVATION AT 40° C.

The object of cultivating the larvae *in vitro* at the body temperature of the final host was to determine whether the sudden increase in temperature alone would provide sufficient stimulus to induce further development and maturation of the genitalia.

(1) *Cultivation in peptone broth*

Within 30 min. of the beginning of incubation in broth in tubes placed in an oven at 40° C., larvae became extremely active and expanded and contracted with such vigour within the tubes that the top of the medium often became frothy and the tubes even vibrated slightly. The bothrial extremity became completely everted and pointed (Text-fig. 2 B) as in the adult form. Cuticular peeling commenced within an hour, and after about 24 hr. cultivation the entire cuticle was shed from the worm. Apart from these features, no noticeable external feature was observed until about 48 hr. later, when eggs began to appear at the uterine pores in the anterior segments containing genitalia. Within a few hours, with the exception of the first eleven segments, every proglottid was ejecting eggs at a rapid rate. The oviposition continued for 2–4 days, the activity gradually diminishing. At the cessation of oviposition the exhausted worms died—the total period of viability *in vitro* under these conditions being 4–6 days.

During oviposition the eggs tended to accumulate in little clusters around the uterine pores, plainly visible as lightish brown patches in the centre of each proglottid containing genitalia. By gentle shaking the eggs were freed and allowed to settle to the bottom of the tubes.

At higher magnification it was further possible to observe not only ejection of the eggs but also the actual sexual process of the cirris—unseen under low power, as the cirris is a very delicate minute structure almost invisible against the white background of the body of the worm. The vigorous undulation of the organism in the medium makes this observation even more difficult, and considerable practice manipulating the light was necessary before it was satisfactorily observed in detail.

Eversion of the cirris commenced at 40–60 hr. after the commencement of incubation. The cirris region became swollen and formed a slight elevation in each proglottid. This swelling later began to 'throb' in a regular manner and finally the cirris everted completely. Once the eversion process started it continued for about 30 hr. During the sexual process, the cirris everted directly outwards; there was no attempt at a sideways movement towards the vaginal pore. The cirris everted and invaginated at the rate of about once per second.

The actual discharge of spermatozoa was not observed, but it can be assumed that they were ejected directly into the culture medium at each eversion, as microscopic examination of the debris at the bottom of the tube revealed their presence. Later experiments with two or three larvae within a culture tube, making cross-fertilization a possibility, gave precisely identical results. Although the worms were frequently in quite close apposition due to confinement within the tube, the cirris always everted directly outwards from the proglottids and in no instance did copulation occur between the worms.

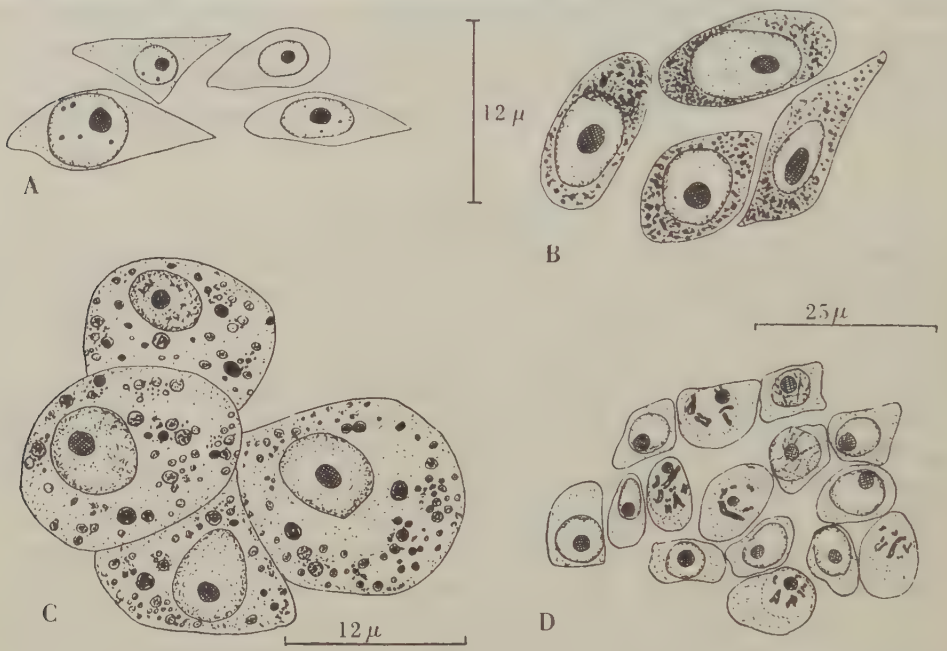
(2) *Histological examination*

Histological examination revealed striking changes in the internal organization with complete maturation of all the organs previously in a primitive immature condition. Worms were fixed at intervals of 24, 48 and 72 hr. cultivation. As the present problem is concerned mainly with the physiological aspects of the development, detailed cytological examination was not undertaken in this investigation.

(*a*) *Female genitalia.* The ovaries show the least degree of change of any of the genitalia. The nuclei, which in the larval stage are either resting or undergoing slow mitotic division, now show meiotic figures especially in the region of the junction with the oviduct. The uterus—narrow and thick-walled in the plerocercoid—becomes greatly enlarged and distended with masses of eggs and free yolk cells (Pl. 2, fig. 5, *ut.*). The yolk glands undergo marked changes. After 24 hr. incubation, the cytoplasm becomes filled with small basiphil granules often accumulated at one end of the cell (Text-fig. 5B). After 48 hr. the cells have become fully developed (Text-fig. 5C) and have increased in size from $9 \times 4\mu$ to $13 \times 10\mu$ (mean values). Numerous globules of yolk of varying sizes and degrees of staining are present in the cytoplasm. The swelling of the yolk cells causes the glands to lie in close apposition so as to appear fused together and form a continuous band around the periphery of each proglottid except in the immediate region of the genital pores (Pl. 2, figs. 3, 5). After Carnoy fixation the fatty component of the yolk becomes dissolved out, so that the granules seen in Text-fig. 5C represent only the protein base of the yolk. In fresh yolk cells, seen in teased-out tissues, the yolk globules appear as yellow refractive spheres which completely fill the cytoplasm. In Champy-fixed material they appear as brownish black globules. No attempt has been made to follow out the origin of the yolk during its formation. One fact, however, is certain, the yolk is formed from the cytoplasmic inclusions and not by nucleolar extrusion, as the nucleolus remains apparently unchanged during vitellogenesis. The eggs as laid varied in size between the limits—lesser diameter 35–46μ, greater

diameter 58–75 μ, with a mean value for ten eggs of $38 \times 64\mu$. These values agree closely with those of Linton (1927), who from preserved specimens obtained 36–42 μ, and 57–69 μ for the lesser and greater diameters respectively.

(*b*) *Male genitalia.* Sections of the testes revealed that complete spermatogenesis had taken place within 40–50 hr. incubation. Every testis capsule contained numbers of mature spermatozoa together with spermatocytes and spermatids in progressive stages of maturation. Spermatogenesis appears normal in every way and follows the typical pattern. A sperm-mother cell by repeated mitotic division gives rise to an 8-celled morula. Meiosis takes place at this stage and results in a 32-celled spermatid morula. The centriole in each spermatid which was not previously visible



Text-fig. 5. A. Resting yolk gland cells in uncultured plerocercoid. B. The same after 24 hr. cultivation in peptone broth at 40° C.; cytoplasm granular. C. The same after 48 hr. cultivation under the same conditions; cytoplasm filled with yolk globules. D. Ovary showing meiosis; cultivation conditions as in C. All figures from Carnoy-fixed material. Heidenhain's haematoxylin.

now appears as a deeply staining granule on the peripheral side of each cell (Pl. 3, fig. 3, *sr.*) at the same time as the nuclei begin to elongate. By further elongation (Pl. 3, fig. 3, *sd.*) the nuclei develop into the typical narrow sperm heads, and the tail arises by outgrowth from the centriole and grows rapidly back. The minute size of the male germ cells makes observations on the chromosomes difficult, but it is tentatively suggested that the haploid number lies between 6 and 8 and the diploid between 12 and 16; these numbers, however, are subject to confirmation.

The mature spermatozoa lie in typical whorls in the lumen of the testis capsules. They have little affinity for nuclear stains and can only satisfactorily be seen in Carnoy-fixed material heavily over-stained in Heidenhain's haematoxylin (Pl. 1,

fig. 2). The vasa efferentia, which are apparently undeveloped in the plerocercoid, are easily visible in the mature worm as very much coiled tubes filled with spermatozoa. The seminal vesicle, which is small in the larva, becomes greatly enlarged and is packed tightly with spermatozoa.

In addition to the normal stages of spermatogenesis described above, the majority of testes contain a very large cell with polypoid chromatin material. These giant cells measure between 10 and 15μ in diameter and contain a very large number of chromosomes—so large, in fact, that it has not been possible to count them in existing material. These cells invariably lie free in the lumen of the testes. There is no evidence to suggest what role they play in the process of spermatogenesis. They may act as 'nurse' cells of some kind, or alternatively they may simply be abnormal cells produced by the artificial conditions of cultivation. Unfortunately, specimens of normally matured adult *Schistocephalus* from the bird host were not available, so that comparison between the normal spermatogenesis and that induced by artificial cultivation was not possible.

(3) *Cultivation in salines*

In contrast with the results obtained in peptone broth, the incubation of plerocercoids in salines or glucose salines did not induce the fully mature condition. In the great majority of cases worms died within 1–3 days after a period of intense activity. Only in one out of the ten larvae used did spermatogenesis and partial vitellogenesis take place. In no case did the cirris undergo eversion, although in the larva in which spermatogenesis had taken place, fully formed spermatozoa were found in the testes. Apart from the partial granulation of the yolk glands in this same larva, indicating that vitellogenesis had commenced, the female genitalia showed no appreciable change. The ovaries showed no meiosis and oviposition did not occur.

(4) *Cultivation of eggs*

After oviposition was completed the mature worms were removed from the media and the tubes gently shaken to stir up the accumulated eggs at the bottom. The broth, together with the eggs, was transferred to a sterile centrifuge tube and centrifuged. The eggs were washed with several changes of sterile water and divided into two portions. One portion was incubated in water in sterile centrifuge tubes in an oven at 25° C.; the other was placed in a few drops of water in a watch-glass in a covered Petri dish exposed to sunlight, after the method recommended by Rosen (1920).

No further development of the eggs was obtained in either case, even after 6 weeks' cultivation. The normal time of development at room temperatures is about 3 weeks (Callot & Desportes, 1934), but in *Ligula* the eggs hatch out in 8 days in the warm summer months (Joyeux & Baer, 1942). Microscopic examination of the eggs revealed that they had undergone no internal development, although the enormous amount of yolk in the eggs of *Schistocephalus* makes observation of the internal changes difficult.

In view of the fact that the worms had not apparently copulated during cultivation, it was at once suspected that the failure of the eggs to hatch was due to non-

fertilization. A careful re-examination of sections of the sexually mature worms showed the absence of spermatozoa from the vagina; nor were any stages that could be interpreted as representing fertilization found. It must be emphasized, however, that on account of their minute size and poor staining affinity, identification of spermatozoa—except in large masses—is one of some difficulty. That fertilization had not taken place was further confirmed, however, by the fact that the ova as seen in sections of the uterus contained apparently the haploid number of chromosomes only.

DISCUSSION

In assessing the suitability of a medium for the artificial cultivation of cestodes *in vitro*, four main criteria must be taken into consideration—viability, normality of behaviour, growth and development.

With the exception of growth, for which there is no definite evidence, peptone broth answers all the other criteria for suitability as a culture medium for both the plerocercoid and the strobilar phase. To some extent, too, even such a relatively simple non-nutrient solution as $\frac{3}{4}$ Locke allows of considerable viability and normality of behaviour, and would be a suitable medium for studying the physiology of the plerocercoid stage, but not the strobilar.

In these empirical investigations, with the exception of the osmotic pressure, physical factors, such as oxygen tension, *p*H, and surface tension, have not been taken into consideration, and further investigation will be required to determine whether they play a significant role in the control of viability. The high viability obtained suggests that, like nematodes, cestodes may be relatively insensitive to a wide variation in environmental factors (Brand & Simpson, 1944). Moreover, throughout the experiments, the media in each tube was only changed every 30 days, and it may be that if the medium was renewed more frequently, considerably higher periods of viability of the plerocercoids could be obtained, as it seems not improbable that the by-products of metabolism may have some cumulative toxic effect.

The viability of the mature *Schistocephalus* (4–6 days) in peptone broth at 40° C. is especially significant, as this period, although apparently brief, is equal to the normal longevity of the adult in the natural bird host; so that, in fact, it is possible to culture the adult *Schistocephalus in vitro* for a period equivalent to its viability *in vivo*.

The fact that plerocercoids can live and behave normally in pure saline media containing no nutrient substance points to a very low metabolic rate in the larval condition. This is not surprising, in view of the fact that for the most part larvae remain motionless within the culture tubes until stimulated by light or heat. Furthermore, since they are undergoing no reproductive processes, the total expenditure of energy will be small. During all this period it is evident that larvae must be drawing on their reserve food supplies. It is well known that the food reserves of cestodes are mainly in the form of glycogen, representing some 20–30% of the dried weight (Brand, 1933; Smorodinzew & Bebeschin, 1935; Wardle, 1937*a*; Reid, 1942). This glycogen is stored in the intercellular spaces in the parenchyma (Ortner-Schönbach, 1913; Wardle, 1937*a*). Many workers believe that glucose is

the main carbohydrate requirement of cestodes, and that glycogen reserves are built up from the absorbed glucose. Wardle (1937*a*) determined chemically the glycogen content of isolated proglottids of *Moniezia expansa* before and after immersion in saline solutions containing polysaccharides. The most significant result was obtained with Tyrode solution plus glucose, in which proglottids immersed for 6 hr. showed a glycogen gain of 25 mg./g. of fresh weight. Wardle suggested that the glycogen served as a 'source of fuel for the longitudinal musculature when the latter is maintaining the condition of muscular tonus which characterizes the normal worm'.

In the light of these results it is interesting to note that the viability and period of normal behaviour of the plerocercoids of *Schistocephalus* was considerably lower in glucose salines than in the corresponding pure salines, with the exception of one worm in normal Locke plus 1% glucose. However, for the most part the range of viability of the larvae in glucose salines and pure salines was so very considerable that it is not possible to treat the figures statistically to obtain a quantitative result for the addition of glucose. It is advisable, therefore, at this stage not to draw any very definite conclusion from these experiments with glucose salines, as there are undoubtedly many other viability factors as yet unconsidered, and only when all these factors are known and carefully controlled will a quantitative determination be possible. It is certain, too, that there will be some natural degree of variation between individual larvae, but as already pointed out, the results in general are comparable with the range of variation of the viability of parasitic nematodes during cultivation *in vitro* (Brand & Simpson, 1942).

The behaviour of larval *Schistocephalus* in salines is in general agreement with the findings of Wardle (1934) for larval cestodes, and of Child and his school (1924, 1926) for free-living platyhelminths. The process of degeneration after prolonged cultivation is similar to that described by Wardle for the plerocercoids of *Diphyllobothrium* in oligoseptic sublethal media. Degeneration commences at the abothrial segment and extends forwards. According to Wardle, this is due to the fact that the region of maximum activity (in this case the bothrial end) is inhibited first by the penetration of the ions H′, K′ and Na′, which induce tonic muscular contraction, and disintegration then commences at the region farthest away from the inhibited region towards which it gradually extends.

The very high degree of natural behaviour and viability shown in $\frac{3}{4}$ Locke with an osmotic pressure ($\Delta = -0{\cdot}42^\circ$ C.) approaching that of *Schistocephalus* as determined by experiment ($\Delta = -0{\cdot}44 \pm 0{\cdot}02^\circ$ C.) suggests that the osmotic pressure is an important factor to be considered in the choice of a culture medium. The osmotic pressure of fresh-water teleosts is in the region of $\Delta = -0{\cdot}54^\circ$ C. (Scott, 1916), and it might be expected that media with an osmotic pressure approaching this figure might more nearly reproduce the conditions in the coelomic cavity of the fish. With salines, at least, this is not the case, as activity was much more marked in $\frac{3}{4}$ Locke than in normal Locke ($\Delta = -0{\cdot}56^\circ$ C.). In peptone broth ($\Delta = -0{\cdot}56^\circ$ C.), however, the duration of natural behaviour was considerably longer than in $\frac{3}{4}$ Locke, but in this case nutritional factors are also concerned, and it is not justifiable to compare the media on the basis of osmotic relationship alone. It is interesting to note

that the osmotic pressure of bird tissues ($\Delta = -0{\cdot}56°$ C., Scott 1916) is close to that of fresh-water teleosts, and thus when the plerocercoids of *Schistocephalus* are taken into the alimentary canal of the bird host, the change in the osmotic pressure of the environment will be slight. It is possible that a study of the osmotic pressures of cestode larvae and that of their hosts might throw some light on the problem of host specificity, although this does not exclude other factors.

It is difficult to decide whether growth of the larva is actually taking place during the cultivation at room temperature, owing to the limitations in the means of measurement employed. Sections revealed that although the cells of the testes and ovaries sometimes show mitosis, no actively dividing somatic cells were found throughout the entire length of the larvae examined. The only reliable criteria of growth are either increase in weight or number of proglottids, and further technical difficulties will have to be overcome before it will be possible to measure these with the required degree of accuracy, and the question of growth finally decided.

The result of incubating the plerocercoids of *Schistocephalus* at 40° C. raises a number of interesting questions. Since full development resulting in oviposition took place only in nutrient broth and not in saline or glucose-saline media, it can be inferred that although the sudden rise in temperature undoubtedly provides the stimulus for further development, complete maturation can only be reached providing sufficient nutriment is available in the medium. This is not surprising, in view of the fact that the eggs of the pseudophyllidean cestodes are very rich in food reserves in the form of yolk. The latter provide nourishment for the ciliated embryo during its free-swimming existence. The food requirements of the worm during incubation at 40° C. must therefore be of a high order, as in addition to the production of yolk, fuel must be provided to maintain the great muscular activity which takes place at this high temperature. In pure salines the helminth must rely on its own natural food reserves which evidently are not quite sufficient to fulfil the requirements at this high metabolic rate. On this account it is difficult to account for the fact that one specimen of *Schistocephalus* underwent complete spermatogenesis and partial vitellogenesis in pure $\frac{3}{4}$ Locke, whereas the remainder showed no development. It is possible, of course, that this specimen may have carried away an excessive amount of coelomic fluid when it was being removed from the fish. It is remarkable that Joyeux & Baer (1942) failed to obtain spermatogenesis in their experiments with *Ligula in vitro* at 38–40° C., although a wide range of nutrient media were employed.

There seems little doubt that the infertility of the eggs is due to the failure of normal copulation to take place. Under artificial conditions of cultivation, the spermatozoa are apparently ejected directly into the culture media and fail to make their way to the vaginal pore. It is possible that there may be some sucking action on the part of the vagina, as the rapid eversion of the cirris was always accompanied by a peculiar jerky movement of the genitalia visible in outline. According to Gamble (1897) and Faust (1939), self-fertilization of each proglottid is the usual procedure in cestodes, but cross-fertilization from one worm to another or from one proglottid to another in the same worm is not an infrequent occurrence. With

the vertical erection of the cirris, it is difficult to see how self-fertilization of a proglottid can take place since the male and female genital pores are separate, and it seems likely that cross-fertilization must be the normal copulatory procedure in *Schistocephalus*. No copulation, however, was observed between two or three larvae cultured in the same tube, which suggests that copulation will only take place when the worms are in *very* close apposition, a condition presumably in which they find themselves in the alimentary canal of the bird host. Due to the hibernation of the fish during the winter months, it has not been possible to obtain additional material to test this hypothesis further.

From purely morphological grounds, the Ligulinae have long been regarded as 'primitive' cestodes. The fact that sexual maturity can be induced by the raising of the temperature in a suitable medium indicates that from the point of view of their physiology also, they are relatively unspecialized. This would account for their occurrence in a variety of final hosts. In most cyclophyllidean cestodes (e.g. *Taenia saginata*), on the other hand, there can be no question of the change in temperature being responsible for providing the stimulus for the transformation of the larval stage into the sexually mature strobila, as the intermediate hosts are also warm-blooded, so that other factors must operate. De Waele (1934) has suggested that the stimulus for the development of cysticercoids is provided by the digestive juices in the final host and that the cuticle of the cestodes serves to protect the worms from digestion, but only in the specific host or closely allied species. In veiw of this hypothesis, it is of interest to note that Joyeux & Baer (1942) obtained sexually mature strobila of *Ligula* by feeding the plerocercoids to ducks, gulls, dogs and cats, but failed to obtain development with rabbits, macaque monkeys and man. Since the body temperatures of these animals are not significantly different, it must be concluded that the physiological conditions of the juices of the gut also play some part in the development of the pseudophyllidean cestodes.

One of the most interesting features of the life cycle of *Schistocephalus* is that although its sexual development is virtually at a standstill when in the plerocercoid phase, it can nevertheless undergo considerable growth in body size. This is proved by the fact that larvae with 60–80 proglottids can be obtained from the fish. Why the somatic cells continue to grow, while the germ cells stop after a certain stage, is a question to which no satisfactory answer can as yet be given.

The present experiments have been relatively successful in the attainment of asepsis, prolonged viability and maturation of the genitalia *in vitro*, but it must be emphasized that they have been only preliminary and necessarily empirical in nature. Further work will be required to determine the factors concerned in the growth of the larval phase and the fertilization of the ova. The information and experience gained, however, will enable more extensive investigations to be undertaken with regard to many problems of paramount importance concerning the metabolism and general physiology of cestodes.

My thanks are due to Professor Spaul for advice and encouragement and to Dr Baylis for help with the literature.

SUMMARY

A technique has been elaborated that enabled the plerocercoid larvae of *Schistocephalus solidus* to be removed from the body cavity of *Gasterosteus aculeatus* without bacterial contamination. Larvae were cultured in plugged test-tubes under completely aseptic conditions in a variety of balanced salines, glucose salines and nutrient peptone broth.

The most successful results were obtained with peptone broth at room temperatures (16–19° C.) in which plerocercoids remained active and showed normal behaviour for periods up to 300 days. In $\frac{3}{4}$ strength Locke's solution, which was found by experiment to be approximately isotonic with *Schistocephalus* ($\Delta = -0{\cdot}44 \pm 0{\cdot}02°$ C.), the mean period of normal behaviour was 114 days. In the remaining saline and saline-glucose media, the mean viability and period of normal behaviour was considerably less.

In the plerocercoid, histological examination revealed that the genitalia are in an immature condition. During cultivation at room temperatures, the genitalia remained in this undifferentiated condition and showed no signs of undergoing spermatogenesis, oogenesis or vitellogenesis.

Plerocercoids were induced to develop into sexually mature adults by raising the temperature of cultivation in peptone broth to 40° C. (i.e. the body temperature of the final host in the natural life cycle). Oviposition took place after 48–60 hr. at this temperature, and histological examination revealed that spermatogenesis, oogenesis, vitellogenesis and shell formation had taken place in a normal manner. The viability of artificially matured *Schistocephalus* was 4–6 days *in vitro*—a period equivalent to the viability of the adult *in vivo*.

The eversion of the cirris was observed in each proglottid after 40 hr. cultivation at 40° C. During the sexual process the cirris everted and invaginated at the rate of about once per second. Cross-fertilization between segments of the same worm or with segments of another worm was not observed. Except for one specimen in $\frac{3}{4}$ strength Locke's solution which underwent spermatogenesis and partial vitellogenesis, larvae cultured in salines or glucose salines at 40° C. died within 1–3 days without further development.

Attempts to hatch out the eggs produced by the cultivation of larvae in peptone broth at 40° C. proved unsuccessful. Histological examination revealed that spermatozoa had not been taken into the vagina. It was concluded that the eggs were not fertilized owing to the failure of normal copulation to take place.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Whole mount of plerocercoid of *Schistocephalus solidus* showing immature genitalia; bothrial segment everted during fixation. Corrosive acetic; borax carmine. × 2·5.

Fig. 2. Transverse section of mature testis heavily overstained to show spermatozoa. Cultured in peptone broth at 40° C. for 48 hr. Carnoy; Heidenhain's haematoxylin. × 1000.

Fig. 3. Longitudinal section of cuticle showing cuticular 'hairs'. Formalin; Delafield's haematoxylin and eosin. × 1000.

Fig. 4. Eggs as seen in section of uterus. Cultured in peptone broth at 40° C. for 48 hr. Carnoy; Heidenhain's haematoxylin. × 1000.

PLATE 2

Fig. 1. Transverse section of infected stickleback showing plerocercoids in the body cavity. Bouin; decalcified; Delafield's haematoxylin and eosin. × 18.

Fig. 2. Transverse section of normal uncultured plerocercoid in region of cirris. Bouin; Delafield's haematoxylin and eosin. × 15.

Fig. 3. Transverse section of same region as fig. 2 after 48 hr. cultivation in peptone broth at 40° C. Seminal vesicle swollen with spermatozoa, testes enlarged. Carnoy; Heidenhain's haematoxylin. × 28.

Fig. 4. Transverse section of normal uncultured plerocercoid in region of uterus and vagina. Yolk glands small and inactive. Bouin; Delafield's haematoxylin and eosin. × 15.

Fig. 5. Transverse section of same region as fig. 4 after 48 hr. cultivation in peptone broth at 40° C. Uterus packed with eggs; yolk glands swollen and active. Carnoy; Heidenhain's haematoxylin. × 28.

PLATE 3

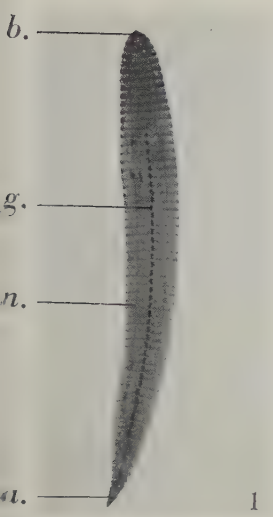
Fig. 1. Transverse section of inactive yolk glands in normal uncultured plerocercoid. Carnoy; Heidenhain's haematoxylin and orange G. × 1000.

Fig. 2. Transverse section of yolk glands after cultivation in peptone broth for 48 hr. at 40° C. Cytoplasm swollen and granular. Carnoy; Heidenhain's haematoxylin. × 1000.

Fig. 3. As Pl. 1, fig. 2 but not overstained; spermatozoa barely visible; shows sperm morula at different stages of differentiation. Carnoy; Heidenhain's haematoxylin. × 1000.

Fig. 4. Transverse section of testis of normal uncultured plerocercoid containing undifferentiated spermatogonia. Carnoy; Heidenhain's haematoxylin. × 1000.

Abbreviations: *a.* undeveloped posterior tip; *b.* bothrial segment; *c.* cirris; *cp.* cuticular processes; *ct.* cuticle; *ex.* excretory canal; *g.* genitalia; *gt.* gut of fish; *h.* cuticular 'hairs'; *n.* nerve cord; *o.* ovary; *p.* plerocercoids in body cavity; *sg.* spermatogonia; *sd.* late spermatid morula; *sp.* mature spermatozoa; *sr.* early spermatid morula; *st.* spermatocyte morula; *sv.* seminal vesicle; *t.* testes; *up.* uterine pore; *ut.* uterus; *v.* vagina; *y.* yolk cell; *yg.* yolk glands; *yu.* yolk cells free in uterus.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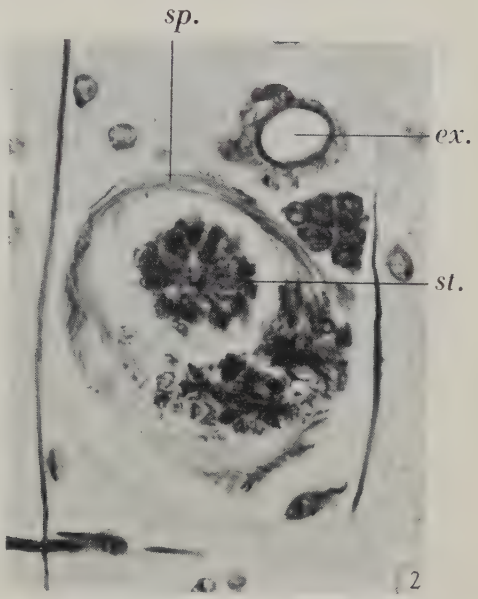
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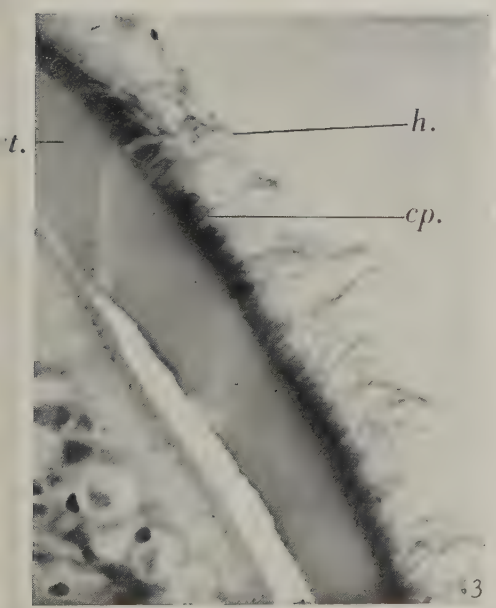
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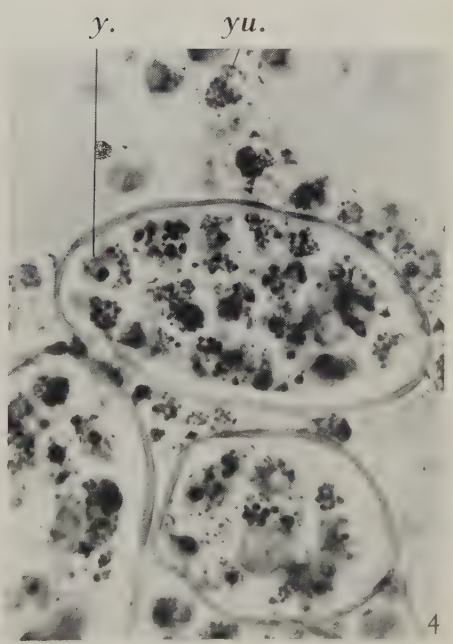
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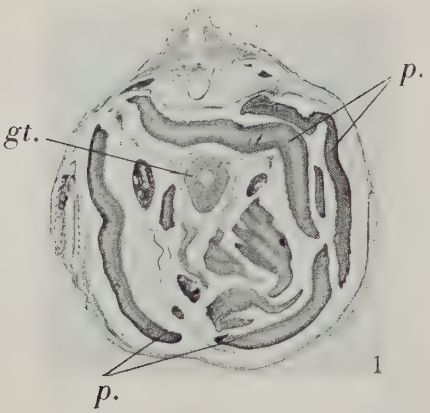
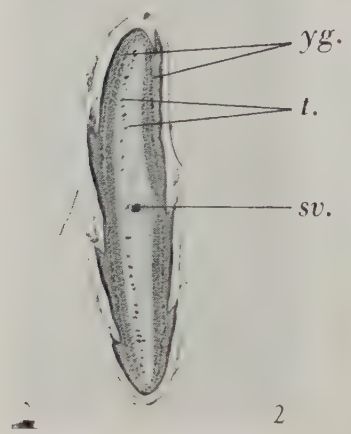
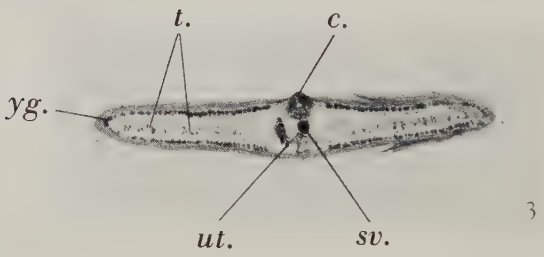
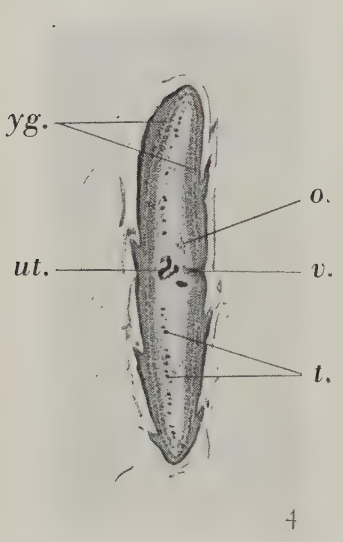
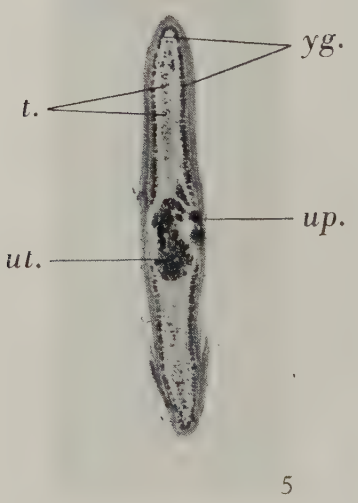


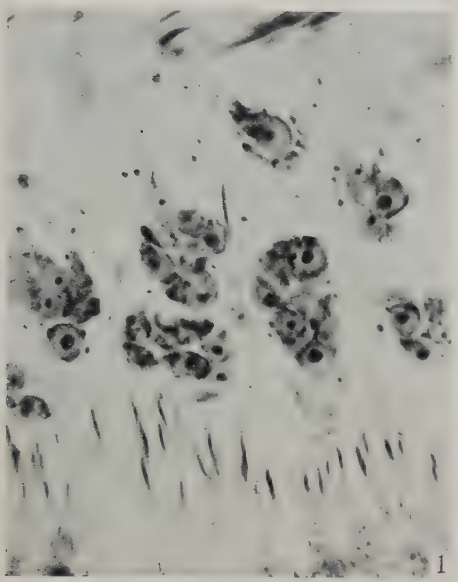








SMYTH—STUDIES ON TAPEWORM PHYSIOLOGY



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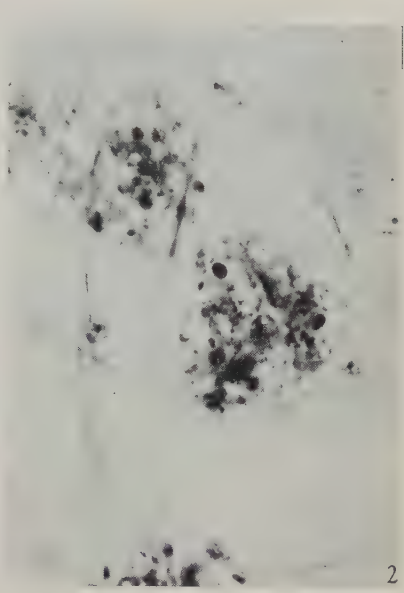

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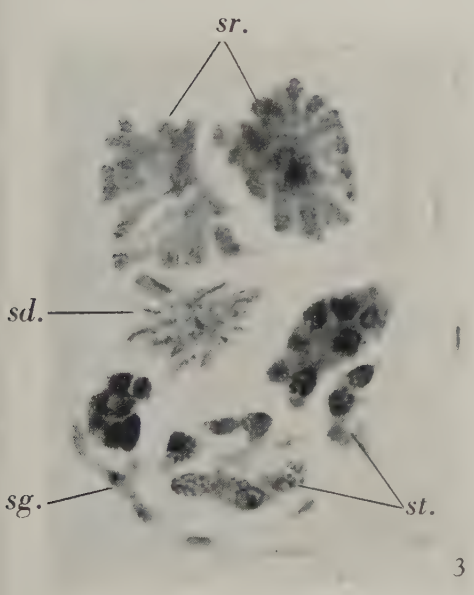

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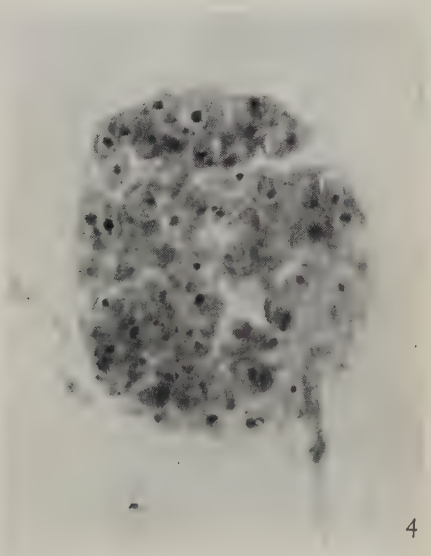

4

THE EFFECTS OF LOW ATMOSPHERIC PRESSURE ON THE FERTILITY OF MALE RABBITS

By ARTHUR WALTON, *School of Agriculture, Cambridge* AND W. URUSKI, *Institute of Animal Pathology, Cambridge*

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INTRODUCTION

The influence of high altitude upon fertility was noticed a long time ago. Monge (1943) quotes the Foundation Charter of Lima in which it is stated that in 1535 the capital of Peru was transferred from Janja (11,500 ft.) to Lima (sea-level) because horses, fowls and pigs did not reproduce there. He also recalls Father Catancha's observation (1639) that the Spanish conquerors did not have offspring at Potosi, Bolivia (14,000 ft.) until 58 years after the city was founded.

Monge (1942, 1943) reports changes in the *p*H of the semen and motility of the spermatozoa, and 50% sterility in unacclimatized rams in the Andes. The fertility of the female at high altitude is unaffected.

Dohan (1942) and Gordon, Tornetta, D'Angelo & Charipper (1943) found a decrease in the testis weight and degenerative changes in the testes of rats exposed to low atmospheric pressure.

The following experiments were designed to investigate the effect of low atmospheric pressure on spermatogenesis in rabbits, using the technique of semen collection described by Macirone & Walton (1938) so that a continuous record could be kept of sperm production, and testicular activity could be studied at all stages of treatment and recovery without having to kill the animal. This method also allows an assessment to be made of sex drive and male potency. Artificial production of low atmospheric pressure in the laboratory does not of course reproduce all the conditions of life at high altitude. In these experiments we are concerned only with the effects of lowered atmospheric pressure.

MATERIAL AND METHODS

Seventeen sexually mature rabbits of different strains, fed on a ration of crushed oats and bran, kale, cabbage and hay *ad lib.* were kept in an air chamber at different reduced pressures for various periods of time. The air was removed from the chamber by a vacuum pump, running continuously and so supplying the animals constantly with fresh air. There was, therefore, no accumulation of CO_2, moisture or other gaseous products of metabolism. The desired pressure was obtained by regulating the intake through a valve. The chamber was connected with a mercury manometer. When the animal was placed in the chamber or removed, the pressure was lowered or raised at a rate of about 20 mm. Hg/min. The rabbits were weighed once weekly. Early in the experiment a marked drop of body temperature was

noticed, and from then onwards the temperature of each animal was taken just before placing it in the chamber and immediately after it was removed.

At least 2 weeks before beginning the experiment the rabbits were trained to serve an artificial vagina. Semen was collected twice weekly throughout the whole period of observation, using the technique described by Macirone & Walton (1938). Immediately after collection the ejaculate was diluted with saline (0·9% NaCl) to 10 ml. In each ejaculate, the motility, the number of spermatozoa and spermatogenetic cells, and the percentage of abnormal spermatozoa were measured. If the first ejaculate was poor a second was collected and the better of the two examined. For examining motility two drops of semen were placed on a slide covered with a cover-glass and examined under $\frac{1}{2}$ and $\frac{1}{6}$ in. objectives and recorded as follows: aspermia = 000, azoospermia = 00, necrospermia = 0, 1–10% of motile spermatozoa = 1, 11–20% = 2, and so forth.

The number of spermatozoa was estimated by means of the haemocytometer (Walton, 1927). Usually 16 squares were counted, but if these included less than 100 spermatozoa, 32 or 48 squares were counted. The standard error of a haemocytometer count is given by the relation $s = \sqrt{n}$, where n = the number counted. The standard errors of the counts in this experiment were therefore approximately $s = \sqrt{100} \leqslant 10\%$. After estimation of the number of spermatozoa and spermatogenetic cells, the abnormal spermatozoa were counted in the same squares and the percentage of abnormal spermatozoa was calculated.

Abnormal spermatozoa fell into six different categories:

(1) Undifferentiated cells of testicular origin in excess of the normal 3%.

(2) 'Immature' spermatozoa, i.e. differentiated spermatozoa but with protoplasmic residues round neck and middle piece.

(3) Spermatozoa with malformed heads.

(4) Spermatozoa with malformed middle piece.

(5) Spermatozoa with malformed tails.

(6) Spermatozoa without tails.

Usually the percentage of abnormals was estimated by inspection of unstained films, but more accurate determinations were made from time to time by the following procedure. Three drops of diluted semen were smeared on a glass slide and dried in air. The cells were then fixed in 10% formol saline for 5 min. followed by hexamethyl violet for 2–3 min. They were then washed in running water, dried, and mounted in Canada balsam.

Examination of the semen of the experimental animals was begun at least 2 weeks before the exposure to low pressure, and was continued until 2 weeks after the semen had returned to normal. Some rabbits were killed after exposure and the testes, epididymides and vasa deferentia were immediately dissected out. One testis, with epididymis and vas deferens, was fixed in Bouin's solution, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examined. From the other testis the epididymis and vas deferens were dissected out separately, cut in small pieces, suspended in 10 ml. saline, and the motility of spermatozoa, the number of spermatozoa and the percentage of abnormal forms were measured.

EXPERIMENTAL RESULTS

Table 1 summarizes the experimental treatments and the main results. Animals subjected to a reduced pressure of between 380 and 400 mm. Hg for 22 hr. daily and up to 7 days' exposure showed no effects of the treatment. They maintained a normal body weight, ate well, copulated readily with the dummy rabbit, and produced ejaculations of normal semen both during the period of treatment and for some time after.

Table 1. *Summary of treatments and results*

Pressure mm. Hg	No. of male	Hr. daily exposure	No. of days exposed	Average fall of body temp. °C.	Maximum % loss of body weight	% of normal no. of sperms*	% abnormal sperms*	Motility of sperms*
380–400	1	22	5	None?	None?	Normal (100%)	Normal (10–20)	Normal (9–7)
	2	22	5	,,	,,	,,	,,	,,
	5	22	6	,,	,,	,,	,,	,,
	6	22	6	,,	,,	,,	,,	,,
	3	22	7	,,	,,	,,	,,	,,
	4	22	7	,,	,,	,,	,,	,,
260–280	10	6	14	−1·3	8·5	52	59	1·5
	693	6	14	−0·4	7·1	64	93	1·8
210–260	11	16	3	−2·7	8·7	Normal	Normal	Normal
	792	16	3	−4·9	7·5	,,	,,	,,
	790	16	6	−2·6	23·2	33	79	1·2
	696	16	11	−3·2	16·6	38	100	0·0
	793	16	11	−3·2	25·8	18	97	0·3
						Males killed for autopsy		
						Vas	Epididymis	Testis
	14	16	1	−3·8	9·4	Normal	Normal	Normal
	864	16	3	−1·8	20·3	,,	,,	Slight effect
	13	16	5	−3·0	21·4	,,	,,	Marked effect
	12	16	6	−2·8	26·3	,,	Few coils empty	Very marked effect

* Mean of four recordings during period of severest symptoms.

With more severe reduction of pressure, symptoms of discomfort of the animals appeared. The chamber was cylindrical and fitted with glass ends so that the animals could be observed during exposure. When the pressure was lowered, the animals became excited at first and respiration was rapid and shallow, but later it became slow and deep. Cyanosis was observed, but it passed off soon after the animals were removed from the air chamber. On removal, the animal's temperature was taken and found to be below normal. The animals appeared sleepy. Sexual desire was slightly diminished, but all animals attempted to mate with the dummy when it was presented, although some did not ejaculate. During the period of exposure there was some loss of body weight which in one case reached 26%. In some animals there was some appearance of acclimatization, the symptoms of distress in

the chamber becoming less severe as treatment continued, but as exposures were not longer than 14 days the full effects of acclimatization were probably not seen. The effect upon the spermatozoa was most marked and appeared in about 10–14 days' time. The total number of spermatozoa in the ejaculate decreased, but an even

Table 2. *Records of individual males before, during and after treatment. Records taken twice weekly. During the fortnight when the symptoms are most severe the figures are printed in heavy type*

Rabbit 10			Rabbit 693			Rabbit 696			Rabbit 793			Rabbit 790		
Motility	Total no. millions	% abnormals	Motility	Total no. millions	% abnormals	Motility	Total no. millions	% abnormals	Motility	Total no. millions	% abnormals	Motility	Total no. millions	% abnormals
8	135	17	8	83	10	7	35	11	9	123	12	9	218	7
8	92	9	8	84	6	7	26	7	7	151	9	8	143	14
8	141	2	8	91	19	7	45	22	8	108	12	8	206	11
8	136	7	8	80	11	8	37	21	8	146	8	9	267	9
Treatment started														
260–280 mm. Hg 6 hr. daily 14 days			260–280 mm. Hg 6 hr. daily 14 days			210–260 mm. Hg 16 hr. daily 11 days			210–260 mm. Hg 16 hr. daily 11 days			210–260 mm. Hg 16 hr. daily 6 days		
8	147	9	8	113	19	8	31	22	—	—	—	—	—	—
8	132	8	7	85	16	—	—	—	—	—	—	—	—	—
8	63	10	7	101	15	—	—	—	—	—	—	Treatment stopped		
7	37	9	8	46	10	Treatment stopped			Treatment stopped			8	230	24
Treatment stopped			Treatment stopped			7	34	52	6	170	15	1	147	83
8	147	14	6	70	29	0	7	90	4	26	41	**1**	**36**	**75**
2	**127**	**39**	6	24	33	1	2	90	**0**	**16**	**90**	**1**	**31**	**85**
1	**19**	**56**	5	11	53	0	1	91	**0**	**12**	**99**	**2**	**64**	**74**
1	**44**	**74**	2	35	87	0	6	100	**0**	**16**	**100**	**1**	**77**	**78**
2	**73**	**67**	**2**	**44**	**96**	0	3	100	**1**	**27**	**97**	5	128	57
3	99	53	**2**	**65**	**93**	**00**	**4**	**100**	4	31	92	6	83	57
6	74	32	**2**	**42**	**63**	**00**	**5**	**100**	3	53	63	6	156	34
6	120	29	**1**	**64**	**91**	**00**	**17**	**100**	3	48	79	7	142	23
3	37	53	3	59	84	**00**	**28**	**100**	5	97	47	7	196	19
5	81	34	5	106	48	00	26	100	5	79	43	7	264	14
4	32	38	7	85	37	1	43	94	4	49	57	8	196	11
6	89	42	6	103	25	1	24	96	6	167	32	8	258	12
7	204	27	4	41	56	3	31	78	6	121	31	8	360	9
8	75	21	7	65	33	1	58	65	7	103	17	8	235	8
7	65	19	6	185	24	3	33	70	7	106	12	8	318	9
8	56	18	7	144	24	3	47	72	8	129	12	8	247	10
8	64	16	8	112	20	5	56	56	8	171	11			
7	58	19	8	83	23	7	37	35						
8	91	12	7	72	21	6	33	38						
8	164	10	8	60	15	7	87	28						

more striking change was the decrease in motility and the increase in abnormal forms. Complete records of some of the more interesting examples are shown in Table 2.

After treatment was stopped all animals made complete recoveries, and after some delay the semen gradually returned to normal. Of the animals killed for autopsy

after exposure to a pressure of 210–260 mm. Hg. for 16 hr. daily, no. 14 exposed for 1 day only showed no changes in the testicular tubules. This degeneration was more marked in no. 13 exposed for 5 days and was very severe in no. 12 exposed for 6 days. In the last example, very few spermatozoa were present in the tubules, but there were many undifferentiated spermatids or spermatocytes and the germinal epithelium was reduced to a few cells in depth. These degenerative changes were more marked in the tubules in the centre of the testis and less marked at the periphery. The rest of the tract appeared normal, though some of the coils in the caput epididymis were devoid of spermatozoa.

DISCUSSION

The experimental results show that low atmospheric pressure has a decidedly harmful effect upon the fertility of male rabbits. Degenerative changes in the testis appeared after 3 days exposure to pressure of 210–260 mm. Hg for 16 hr. daily (Table 1, no. 864), and effects were noticeable in the ejaculated spermatozoa after about a fortnight in all animals affected (Table 2). This delay in the appearance of abnormal spermatozoa in the ejaculate indicates that the spermatozoa in the vas deferens and lower sections of the epididymis were not affected, and that the harmful effect is upon the spermatozoa in the testis, either during spermatogenesis or spermiogenesis. The delay represents the time taken for the passage of spermatozoa through the epididymis when collections are taken twice weekly as in these experiments. Prolonged exposure (11 days) resulted in one animal (Table 2, no. 696) becoming completely sterile for a period of over a month. A short exposure to 210–260 mm. Hg for 3 days did not noticeably affect the spermatozoa in the ejaculate (nos. 11 and 792). A less severe reduction of pressure (260–280 mm. Hg applied for 6 hr. a day for 14 days) had a significant but less marked effect upon the spermatozoa. With pressure reduced only to 380–400 mm. Hg no abnormal symptoms appeared in animals exposed for 22 hr. daily for up to 7 days (Table 1, nos. 1–6).

From these experiments it is clear that both severity of treatment and length of exposure contribute to the production of sterility. It is also noticeable that the effect is chronic and the animal takes a fairly long time to recover, although eventually it does so completely.

The histological evidence certainly indicates that the effect is upon the spermatozoa in the testis, and mainly during the phase of spermiogenesis. This is also confirmed by the semen studies. The number of spermatozoa ejaculated falls, but is never in complete abeyance, showing that spermatogenesis continues although at a low level. The motility of the cells, however, is very markedly affected, and there is a very great increase in abnormal forms, mainly consisting of undifferentiated spermatids or spermatocytes. The hormonal function of the testis does not appear to be affected in any way. Even during the period of most extreme treatment the animals copulated readily with the dummy when it was introduced into the cage. It is true that some of them failed to ejaculate, but this may have been due to physical debility rather than to lack of sexual drive.

The most obvious explanation of the effect of low pressure is to attribute it to oxygen deprivation, acting directly upon the tissues concerned. This has some support from the histological finding that spermiogenesis is affected more severely than spermatogenesis. The cells in the centre of the tubules are farther from the blood supply than the germinal epithelium. Secondly, it was noticed that tubules in the centre of the testis itself were more affected than those nearer the peripheral arterial supply. Campbell (1935), Williams & Smith (1935), and Patterson, Smith & Pickett (1938) found a loss of fertility in animals in which anoxemia was produced by causing them to breathe air containing carbon monoxide.

A less direct mode of action is also possible. Armstrong & Heim (1938) draw a parallel between the symptoms of mountain sickness and Addison's disease, and suggest that the former may be due to cortico-adrenal insufficiency. Pincus & Hoagland (1943) report an increase in 17-ketosteroids in the urine of animals exposed to low atmospheric pressure. Gordon *et al.* (1943) found that the potency of the gonadotropic hormone of the pituitary glands of rats exposed to low atmospheric pressure is significantly greater than in normal rats. One cannot, therefore, at this stage, exclude the possibility that the effect upon the testis is an indirect one and due to hormonal disturbance.

SUMMARY

1. Low atmospheric pressure has a detrimental effect upon the fertility of male rabbits.

2. Changes can be detected in semen samples and by histological examination of the testis.

3. The changes induced in the testis take a chronic course but are completely reversible on returning the animal to normal atmospheric pressures.

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THE THEORY OF ALTERNATING CURRENT MEASUREMENTS IN BIOLOGY AND ITS APPLICATION TO THE INVESTIGATION OF THE BIOPHYSICAL PROPERTIES OF THE TROUT EGG

By LORD ROTHSCHILD

From the Department of Zoology, Cambridge

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(With Eight Text-figures)

Alternating current measurements in biology are concerned with the resistance and capacitance of the cell membrane, and the resistance of the cell interior. No systematic electrical measurements have so far been made on the cell nucleus.

Such expressions as resistance, capacitance and impedance are generally associated with wireless sets or wireless components. They have, however, a physico-chemical meaning. The resistance of the cell membrane is a measure of the ability of ions to pass through it, while the capacitance of the cell membrane gives an indication of its dielectric constant which in turn is an indication of chemical composition. The capacitance depends on the distribution of charge on, in, or round the membrane. Substitution or deformation of dipoles, or alteration of the forces controlling the relative positions of charged particles will make the capacitance vary. If the dielectric constant is known or guessed, measurement of the capacitance enables the thickness of the cell membrane to be computed. The equation for the capacitance of a parallel plate condenser summarizes these relationships:

$$C = \frac{\epsilon A}{4\pi l}, \tag{1}$$

where ϵ = dielectric constant, A = cross-sectional area, and l = distance between the 'plates' of the condenser.

The dielectric constant of a polar liquid or solution can be considered as a measure of the number of molecules oriented by an external field of unit strength. As the molecules are oriented by a torque which depends on the external field strength and the dipole moment of the molecular species concerned, and as the orientation is hindered by frictional forces in the solution which are proportional to the rate of orientation, it is evident that the dielectric constant may *not* be a constant but a variable depending on the frequency of the applied external field.

When the source of current in an electric circuit is direct, the relationship between voltage, current and resistance is given by Ohm's law:

$$R = \frac{E}{I}, \tag{2}$$

where R = resistance, E = voltage, and I = current. If the source of electric current is alternating, of the form

$$I = I_0 \sin \omega t, \tag{3}$$

where I_0 = amplitude, $\omega = 2\pi \times \nu$, and ν = frequency, Ohm's law still holds good, but the system does not have a simple resistance R but an impedance Z which includes the resistance and also the reactance X due to any capacitance the conductor may have. The reactance of a condenser is its 'resistance' to the passage of an alternating current. Unlike direct current, alternating current passes through an ideal condenser even though it has an infinite direct current resistance.

The reactance of a capacitative system is inversely proportional to the capacitance and the frequency: mathematically speaking, $X = 1/\omega C$. It is this fact which often makes measurements with alternating current (a.c.) more practical than those with direct current (d.c.).* Suppose, for example, we wish to investigate the resistance of the cytoplasm in a spherical cell. Measurements would be almost insuperably difficult by d.c. methods. The resistance of the cell membrane is usually very high compared with that of the cytoplasm. If measurements were made by means of electrodes outside the cell, the membrane resistance would swamp the cytoplasm resistance. The only alternative method would be to insert two electrodes into the cytoplasm and to flow current through the cytoplasm between them. This procedure is technically too difficult to be successful. The cell membrane has a significant capacitance. If a.c. is used, the impedance of the cell membrane can be varied at will by varying the frequency of the applied current. If we wish to measure the resistance of the cytoplasm, all that it is necessary to do, in principle, is to increase the frequency of the applied current until the membrane impedance is small compared with the cytoplasm resistance. The capacitance of the cell membrane enables the high resistance of the cell membrane to be 'shorted', and the difficulties which are inherent in the d.c. method are obviated. The a.c. technique also obviates difficulties due to the medium surrounding the cell. If this medium has a high conductivity and current has to pass through the cell, the frequency of the applied current is increased until the impedance of the cell membrane is low compared with that of the medium. At high frequencies most of the current passes through the capacitance of the cell membrane; at low frequencies, where the capacitative reactance is greater, more of the current flows through the resistance of the cell membrane, until in the limiting case at zero frequency or with d.c., all the current passes through the membrane resistance. It has previously been stated that $X = 1/\omega C$. If $\nu = 0$, $X = 1/0 = \infty$, and no current passes through the capacitance. At the other end of the frequency spectrum, $\nu = \infty$ and $X = 1/\infty = 0$. All the current passes through the capacitance of the cell membrane which offers no resistance to the passage of current.

The cell membrane may for the moment be represented electrically as a resistance and a capacitance in parallel (Fig. 1*a*). When a.c. is applied across the membrane, the current flow will be distributed between the two branches of the network

* d.c. pulses are considered as a special case of a.c.

according to their respective impedances at the particular frequency used. The impedance of a resistance and capacitance in parallel is given by the expression

$$Z_p = \sqrt{\frac{R_p^2}{1+\omega^2 C_p^2 R_p^2}}, \qquad (4)$$

and, in complex notation,

$$\mathbf{Z}_p = \frac{R_p}{1+j\omega C_p R_p}, \qquad (4{\cdot}1)$$

where $j=\sqrt{-1}$. At a particular frequency ν_j, a network consisting of a resistance and a capacitance in parallel is indistinguishable from a resistance and a capacitance in series (see Fig. 1*b*). The impedance of the latter is given by the expression

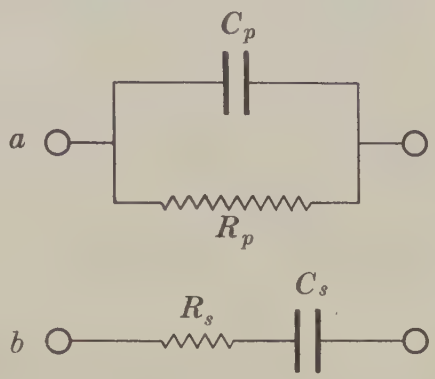

Fig. 1. *a*, resistance and capacitance in parallel. *b*, resistance and capacitance in series.

$$Z_s = \sqrt{\left(R_s^2+\frac{1}{\omega^2 C_s^2}\right)} \qquad (5)$$

and

$$\mathbf{Z}_s = R_s+\frac{1}{j\omega C_s}\,^{*}. \qquad (5{\cdot}1)$$

At the particular frequency ν_j,

$$R_s = \frac{R_p}{1+\omega^2 C_p^2 R_p^2} \qquad (6)$$

and

$$C_s = C_p\left(1+\frac{1}{\omega^2 C_p^2 R_p^2}\right). \qquad (6{\cdot}1)$$

The two networks are indistinguishable and equivalent at a particular frequency.

Electrical networks have a function ϕ, the phase angle, which is an index of the difference between the time when a sinusoidally varying current flowing into the terminals of the network reaches its maximum or any other specified value, and the time when the voltage across the terminals of the network reaches its maximum or any other specified value. In the parallel network

$$\phi = \tan^{-1}\omega C_p R_p. \qquad (7)$$

In the series network

$$\phi_s = \tan^{-1}\frac{1}{\omega C_s R_s}. \qquad (8)$$

If at a particular frequency, $Z_p=Z_s$,

$$\phi_p = \phi_s. \qquad (9)$$

In any electrical network which is not purely resistive, the voltage across the terminals of the network and the current through the terminals are not in phase.

* If $1/\omega C_s$ is written as X_s (the reactance), $\mathbf{Z}_s=R_s+X_s/j$ or R_s-jX_s. This expression will be used later.

The value of ϕ varies with the frequency except in the special case of a network consisting of a pure condenser and other cases referred to later in this paper. The phase angle is constant at all frequencies and equals 90° across the terminals of a pure condenser.

When considering the cell as an electrical network, it is not sufficient to consider the cell membrane alone; the resistance of the cytoplasm and of the external medium must be included. In general these have negligibly small capacitances. An electrical network which is equivalent to a cell or suspension of cells in a conducting medium is shown in Fig. 2. This network is over-simplified in various ways, particularly in that the cell membrane resistance in parallel with the capacitance is omitted. Other omissions will be considered in due course.

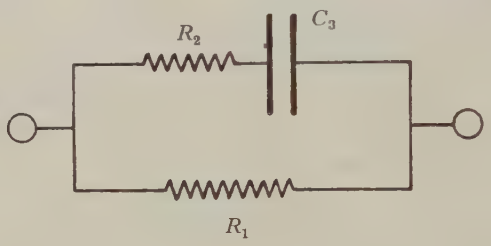

Fig. 2. A simplified electrical network which is equivalent to a spherical cell, or suspension of spherical cells, in a conducting medium. R_1, resistance of conducting medium; R_2, resistance of cytoplasm or cell interior; C_3, capacitance of cell membrane.

This is not the *only* equivalent electrical network. Others could be invented. Its impedance is given by the expression

$$\mathbf{Z} = R_1 \frac{1+\omega^2 C_3^2 R_2(R_2+R_1)-j\omega C_3 R_1}{1+\omega^2 C_3^2(R_2+R_1)^2}. \qquad (10)$$

At a particular frequency, this network is equivalent to a simple parallel network. If it is balanced in an a.c. bridge by a resistance and a condenser in parallel,

$$C_p = \frac{C_3}{1+\omega^2 R_2^2 C_3^2} \qquad (11)$$

and

$$R_p = \frac{R_1(1+\omega^2 R_2^2 C_3^2)}{1+\omega^2 R_2(R_1+R_2)\,C_3^2}. \qquad (11{\cdot}1)$$

In biological a.c. measurements, the material (a cell, suspension of cells, a membrane, etc.) is placed in the unknown arm of an a.c. bridge, balance being effected by a resistance and capacitance in parallel in the standard arm (Fig. 3). The frequency is then varied over as wide a range as possible. From the observed values of resistance and capacitance in the standard arm and from a knowledge of the electrolytic cell constant and the ratio of biological cell volume to volume of external medium plus cell, the various electrical parameters in the biological material can be determined. These parameters are: the capacitance of the cell membrane C_3,

the resistance of the cell membrane R_4, and the resistance of the cytoplasm or cell interior R_2.

How can the observed values of the parallel resistance and capacitance in the standard arm, R_p and C_p, be manipulated to reveal the values of the membrane resistance, the membrane capacitance, and the resistance of the cell interior? A convenient starting-point for the mathematical theory of the conversion is Maxwell's equation for the resistance of a random suspension of spherically packed homogeneous spheres (Maxwell, 1873):

$$\frac{1-r_1/r}{2+r_1/r} = \rho\frac{1-r_1/\bar{r}_2}{2+r_1/\bar{r}_2}, \qquad (12)$$

where r = specific resistance of the whole suspension, r_1 = specific resistance of the suspending medium, $\bar{r}_2$ = specific resistance of the suspended spheres, and ρ = the volume concentration of the spheres. If the volume of the spheres is x and that of the medium is y, $\rho = x/x+y$.

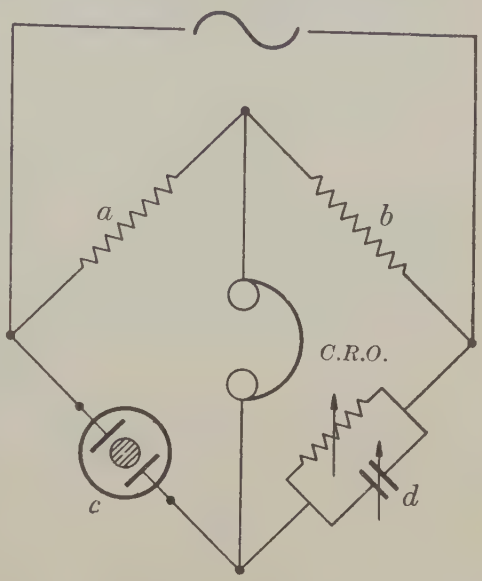

Fig. 3. Alternating current bridge. *a* and *b*, ratio arms; *c*, unknown arm containing egg in electrolytic cell; *d*, standard arm; *C.R.O.* detector.

The biological material is placed in an electrolytic cell in the unknown arm of the a.c. bridge. This electrolytic cell has a cell constant k. The specific resistance of the medium in this cell, r_1, is equal to the observed resistance, $R_1 \div k$. In the following pages lower-case letters represent specific values, and upper-case letters observed values, with one exception which will be discussed in due course.

Maxwell's equation was derived for a suspension of spheres.* We are concerned here with a single sphere. Before proceeding to the development of the equations for biological analysis, it is therefore necessary to establish that the relationship holds good for a single sphere, and under what conditions. Experiments have been done with this end in view. The sphere selected was a glass marble, which may be considered to be homogeneous and as having an infinite resistance $\bar{r}_2$; the suspending medium r_1 was tap water.

If $\bar{r}_2 = \infty$, equation (12) becomes

$$\frac{1-r_1/r}{2+r_1/r} = \rho\frac{1-r_1/\infty}{2+r_1/\infty}. \qquad (13)$$

* A similar expression can be derived for a suspension of cylinders. In this case equation (12) becomes

$$\frac{1-r_1/r}{1+r_1/r} = \rho\frac{1-r_1/\bar{r}_2}{1+r_1/\bar{r}_2}.$$

Solving this equation for ρ,

$$\rho = 2\frac{1-r_1/r}{2+r_1/r}. \tag{14}$$

In experiments with a marble, ρ can be calculated when r_1 and r have been evaluated. (These are measured at one particular frequency only. As no capacitances are involved, they remain approximately constant over a wide frequency range.) ρ can also be calculated by direct observation, as the volume of the marble can be measured and the volume of tap water put into the cell is known. Comparison of the observed and calculated values of ρ is a convenient method of finding out if Maxwell's equation holds good for single spheres and, if so, up to what volume concentrations.

Some results of measurements and calculations of this sort are shown in Table 1. The electrolytic cell used was cubical and made of paraffin wax. Two opposite sides were completely covered by platinized platinum plates, the electrodes. The top of

Table 1

Exp.	Vol. of glass ball (c.c.)	ρ calculated from Maxwell's equation	ρ observed by direct measurement	% difference
1	5·64	0·355	0·376	5·5
2	2·94	0·190	0·196	2·5
3	1·64	0·106	0·109	3·0
4	0·052	0·00346	0·00347	0·25

the cell was covered with a piece of ground glass, the whole being filled with tap water. At the frequencies employed, no electrical conduction takes place through the glass or paraffin wax. Therefore the operative volume of the electrolytic cell is the volume bounded by the platinum electrodes. Four sizes of marbles or glass balls were used.

It is clear from this table that Maxwell's equation holds good up to about 5% for single spheres as well as for suspensions of spheres, and up to high volume concentrations, at any rate when $\bar{r}_2 = \infty$. The highest volume concentration which is possible, i.e. when the inserted sphere touches all sides of the electrolytic cell internally, is 0·523, which may be compared with Exp. 1 in the above table.

Solving Maxwell's equation (12) for r, the specific resistance of the suspension,

$$r = r_1\frac{r_1(1-\rho)+\bar{r}_2(2+\rho)}{r_1(1+2\rho)+2\bar{r}_2(1-\rho)}. \tag{15}$$

This equation assumes that the sphere is homogeneous. A trout egg is not. Nor, for that matter, are any other biological cells. A trout egg is a biphase sphere whose internal resistance is low compared with that of the cell membrane and where a capacitance is present, situated mainly in the cell membrane. In these circumstances we must substitute for $\bar{r}_2$,

$$\mathbf{z}_2 = r_2 + \mathbf{z}_3/a, \tag{16}$$

where $\mathbf{z}_2$ = complex impedance of the cell (instead of the resistance $\bar{r}_2$), $\mathbf{z}_3$ = complex impedance of a unit area of cell membrane, a = radius of the cell in cm., and r_2 = specific resistance of the cell interior. The substitution of $\mathbf{z}_2 = r_2 + \mathbf{z}_3/a$ for $\bar{r}_2$ in Maxwell's original equation depends on the following assumptions (Cole, 1928):

(*a*) that the thickness of the cell membrane is so small that higher powers than the first can be neglected;

(*b*) that the ratio r_2/r_4 is small compared with unity and can be neglected;

(*c*) that current flow in the surface layer of the cell is radial;

(*d*) that the potential gradient inside the cell is uniform.

In (*b*) above, r_4 is the specific resistance of the cell membrane: that is, the resistance of a centimetre cube of the membrane. It is measured in ohm-cm. When, however, r_4 occurs in other parts of this paper, or in others dealing with similar subjects, it refers to the resistance of a square centimetre of membrane and is therefore expressed in ohm-cm.^{2} Capacitances are, however, expressed in μF-cm.$^{-2}$ These differences are intrinsically somewhat confusing, and not made less so by occasional printed references to membrane resistances in ohm/cm.^{2} which are wrong. Furthermore, different authors use different conventions as regards the meanings of upper- and lower-case letters. At present it seems impossible to resolve these differences.

The trout egg differs from many cells in that the protoplasmic or 'living' part of it, the vitelline membrane, is restricted to a thin shell only a few microns thick, surrounding a yolky globulin-containing fluid whose electrical conductivity is said to be equivalent to that of a 0·125 M solution of NaCl (Gray, 1932).

If it is assumed that the vitelline membrane has no resistance in parallel with its capacitance, i.e. that it is non-conducting,

$$\mathbf{z}_3 = \frac{1}{j\omega c_3}, \tag{17}$$

where c_3 = capacitance per unit area of cell membrane. If c_3 has a resistance in parallel with it,

$$\mathbf{z}_3 = \frac{r_4}{1 + j\omega c_3 r_4}, \tag{18}$$

where r_4 = resistance of a unit area of cell membrane. Calculations are much simplified by assuming that the cell membrane is non-conducting, though this assumption would not be justified in a more refined analysis. If $r_2 + \mathbf{z}_3/a$ is substituted for $\bar{r}_2$ in equation (15), the resistance r of the suspension becomes the complex impedance $\mathbf{z}$. Then

$$\mathbf{z} = r_1 \frac{r_1(1-\rho) + (2+\rho)(r_2 + \mathbf{z}_3/a)}{r_1(1+2\rho) + 2(1-\rho)(r_2 + \mathbf{z}_3/a)}, \tag{19}$$

where $\mathbf{z} = r + jx$, r = the resistive part of the total impedance, and x = the reactive part of the total impedance. The resolution of $\mathbf{z}$ into the components r and jx is discussed in greater detail on p. 85.

Solving equation (19) for $r_2+\mathbf{z}_3/a$ and substituting $r+jx$ for $\mathbf{z}$,

$$r_2+\mathbf{z}_3/a = r_1\frac{r_1(1-\rho)-r(1+2\rho)-jx(1+2\rho)}{2r(1-\rho)-r_1(2+\rho)+2jx(1-\rho)}. \tag{20}$$

As it is assumed that the membrane resistance r_4 is infinite, $\mathbf{z}_3/a = 1/ja\omega c_3$. Substituting this in equation (20) and separating into real and imaginary parts:

$$r_2 = r_1\frac{mn-qx(1+2\rho)}{n^2+q^2} \tag{21}$$

and

$$c_3 = \frac{1}{a\omega r_1^2 x}\frac{n^2+q^2}{n(1+2\rho)+2m(1-\rho)}, \tag{21·1}$$

where $m=r_1(1-\rho)-r(1+2\rho)$, $n=2r(1-\rho)-r_1(2+\rho)$, and $q=2x(1-\rho)$.

Equation (21·1) can be simplified to

$$c_3 = \frac{1}{a\omega r_1^2 x}\frac{n^2+q^2}{9\rho}. \tag{21·2}$$

In equations (21) all quantities are observable and the observed values can be converted to specific values where necessary by dividing the observed value by the electrolytic cell constant.* r_1 can be observed in a blank run, i.e. with no cell present, while ρ can be measured directly. r and x are the equivalent series resistance and reactance of the whole suspension, and can be calculated from the measured values of the parallel resistance and capacitance in the a.c. bridge standard arm by means of equations (6) and (6·1).

There is a simpler method of calculating the specific resistance of the cell interior than by equation (21) which does not involve certain difficulties inherent in this equation. At infinite frequency the capacitative part of the cell membrane has zero impedance. In these circumstances a.c. measurements are only concerned with the internal resistance r_2, as the spherical cell becomes a homogeneous sphere of specific resistance r_2. Solving equation (12), for r_2 at infinite frequency,

$$r_2 = r_1\frac{r_1(1-\rho)-r_\infty(1+2\rho)}{2r_\infty(1-\rho)-r_1(2+\rho)}, \tag{22}$$

where r_∞ = specific resistance of the suspension at $\nu=\infty$.

It is not always easy, and sometimes even impossible, to measure ρ. But at zero frequency ρ can be expressed in terms of observable quantities. Returning to equation (12),

$$\frac{1-r_1/r}{2+r_1/r} = \rho\frac{1-r_1/\bar{r}_2}{2+r_1/\bar{r}_2}.$$

At zero frequency this equation becomes

$$\frac{1-r_1/r_0}{2+r_1/r_0} = \rho\frac{1-r_1/\infty}{2+r_1/\infty}, \tag{23}$$

* $r_j=R_j/k$. Therefore R_j/k is substituted for r_j in each case. Equation (21·2) must be multiplied by a factor to correct for c_3 being of the 'polarization' type. This is discussed in detail later.

where r_0 = specific resistance of the suspension at $\nu = 0$. $\bar{r}_2 = \infty$ because $x_3 = \frac{1}{0.c_3} = \infty$.

Solving equation (23) for ρ,

$$\rho = 2\frac{1 - r_1/r_0}{2 + r_1/r_0}. \tag{24}$$

ρ is now in terms of observable quantities as r_0 can be measured by a method similar to r_∞.

Any electrical network consisting of any combination of resistances and condensers can be expressed at a particular frequency in terms of a single resistance and a single condenser in series. The impedance of the latter may be expressed in the form

$$\mathbf{Z} = R_s - jX_s, \tag{25}$$

where $X_s = 1/\omega C_s$ (see footnote on p. 79), R_s is the resistance and X_s the capacitative reactance of the network. Multiplying by j implies multiplication by $\sqrt{-1}$; it also involves the rotation of a line through 90°. In a.c. measurements the unknown is balanced by a resistance and condenser in parallel. At any particular frequency, the electrical network or system in the unknown arm is equivalent to a resistance and condenser in series, and therefore can be expressed in the form $\mathbf{Z} = R_s - jX_s$. If the observed standard arm parallel resistance and condenser values are converted to the form $R_s - jX_s$* and the values of R_s so obtained are plotted along the x-axis and the values of X_s along the $-jx$ (x rotated through 90°) or $-y$-axis, the points so obtained describe a semicircle with the centre of the circle on the x- or resistance-axis (see Fig. 4). Each co-ordinate R_s, $-jX_s$ is the terminal of the impedance vector at that frequency. Therefore if any network consisting of any combination of resistances and one condenser† is expressed in the equivalent form $R_s - jX_s$, the locus of the terminals of the impedance vectors at various frequencies is a semicircle with its centre on the resistance axis.

With certain reservations discussed later, a trout egg in tap water is a combination of resistances and a capacitance. If the values of R_p and C_p which balance it at various frequencies are expressed in the equivalent form $R_s - jX_s$, and the values so obtained plotted in the way described above, the points at the ends of the semicircle, where it cuts the R_s-axis, will be R_∞ and R_0, R_∞ being the one nearer the origin. R_0 and R_∞ are obtained by extrapolation.

In certain physical systems, in the trout egg, and in many other biological cells, the $R_s - jX_s$ plot does not give a semicircle with its centre on the resistance axis, but an arc of a circle, cut by the resistance axis, with its centre above it (see Fig. 5). The half-angle formed by the radii from R_0 and R_∞ to the centre of the circle is the phase angle between current and voltage of the capacitative element in the network

* The standard arm could equally well contain a resistance and a condenser in series. The advantage of the parallel arrangement is that with it, one terminal of the resistance and of the condenser can be at earth potential, thus eliminating hand capacitances, etc.

† If the network contains more than one condenser, this analysis is incorrect.

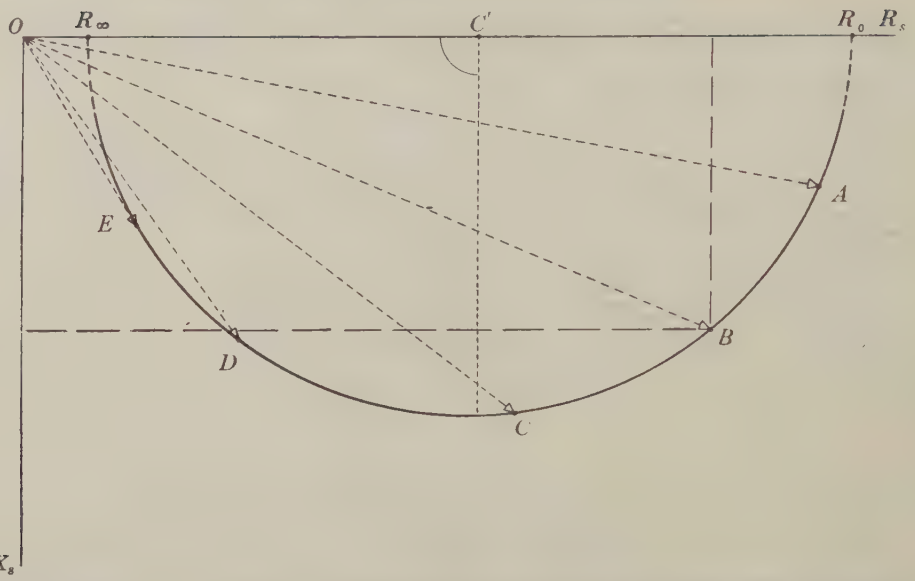

Fig. 4. The unknown arm of the a.c. bridge contains the network shown in Fig. 2. This is balanced at various frequencies A, B, C, D and E by a resistance R_p and a condenser C_p in parallel in the standard arm. The values of R_p and C_p so obtained are converted into the equivalent form $R_s - jX_s$, and the values of R_s and jX_s are plotted on the x- and y-axis respectively. The values at A, B, C, D and E are the terminals of the impedance vectors, OA, OB, etc. The magnitude of the impedance vector OC is $\sqrt{(R_s^2+X_s^2)}$, which is the modulus of $R_s - jX_s$. The centre of the semicircle lies on the resistance axis at C'. As the angle $OC'R_0$ is 180°, the phase angle between current and voltage across the terminals of the ideal condenser in the unknown arm is 90°. R_0 and R_∞ are obtained by extrapolating the semicircle until it cuts the resistance axis (dotted lines).

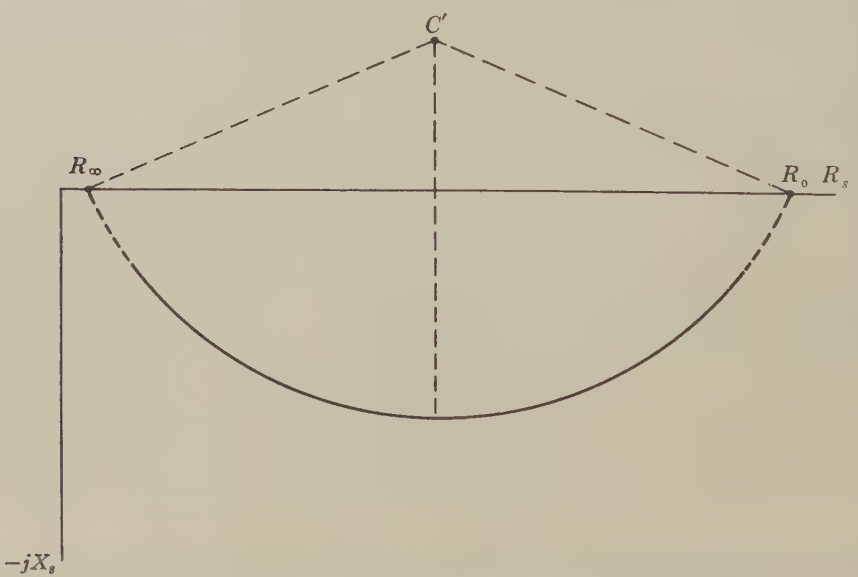

Fig. 5. $R_s - jX_s$ plot for a network containing one polarization capacitance. Note centre of semicircle above the resistance axis.

in the unknown arm. Alternatively, the angle formed by the tangent to the semicircle at R_0 or R_∞ and the resistance axis is the phase angle. Suppose that there is an ordinary electric network consisting of a condenser and various resistances in the unknown arm and $R_s - jX_s$ is plotted in the usual way at various frequencies. The centre of the resultant semicircle will be on the resistance axis; the half-angle subtended by the radii from R_0 and R_∞ at the centre is 90°, which is the phase difference between current and voltage at all frequencies across the terminals of an ideal condenser.* (In an ideal condenser, $\phi = K = \frac{1}{2}\pi$.) By means of the $R_s - jX_s$ plot and from the position of the centre of the resulting circle, certain information is extracted about the capacitative element in any network consisting of any combination of resistances and a capacitative element. That information is either (1) that the capacitative element is an ordinary condenser where $\phi = K = \frac{1}{2}\pi$, (2) that the capacitative element is not an ordinary condenser but is one of a special type in which the phase angle is constant at all frequencies but is less than $\frac{1}{2}\pi$, or (3) that the capacitative element *is* an ordinary condenser but that it is in some way indistinguishably associated with a resistance whose value varies in such a way that the phase angle of the associated condenser and resistance remains constant at all frequencies and less than $\frac{1}{2}\pi$. Alternatively, both the condenser and resistance could vary with frequency, but a variation in capacitance alone will not produce the effect. This capacitative system, where $\phi = K = < \frac{1}{2}\pi$, has been called a variable impedance element, a dielectric impedance element, or a polarization capacitance. No very satisfactory physico-chemical model has so far been devised to reproduce the behaviour of variable impedance elements with constant phase angles.

If the cell membrane is permeable to ions, one cannot assume that the resistance r_4, shunting its capacitance, is infinitely high. The evaluation of the membrane resistance in terms of observable quantities involves measurements at zero and infinite frequency, and measurements of the volume concentration. At zero frequency, the membrane impedance for a square centimetre of membrane, $\mathbf{z}_3$, becomes a pure resistance r_4, because at zero frequency, the impedance of the membrane capacitance is infinite. In these circumstances, Maxwell's equation (equation (12)) becomes

$$\frac{1 - r_1/r_0}{2 + r_1/r_0} = \rho \frac{1 - r_1/(r_2 + r_4/a)}{2 + r_1/(r_2 + r_4/a)}, \tag{26}$$

where r_4 = membrane resistance of a square centimetre of membrane. The other parameters in this equation have their usual significance. Solving this equation for r_4,

$$r_4 = a\left[\frac{r_1^2(1-\rho) - r_0 r_1(1+2\rho)}{2r_0(1-\rho) - r_1(2+\rho)} - r_2\right], \tag{27}$$

or in observable quantities

$$r_4 = \frac{a}{k}\left[\frac{R_1^2(1-\rho) - R_0 R_1(1+2\rho)}{2R_0(1-\rho) - R_1(2+\rho)} - R_2\right]. \tag{27·1}$$

* In a capacitative circuit where $E = E_0 \sin \omega t$, the charge Q on the condenser is $CE = CE_0 \sin \omega t$. $I = dQ/dt$; therefore $I = \omega C E_0 \cos \omega t = I_0 \sin(\omega t + \frac{1}{2}\pi)$. The current leads the voltage by 90°.

From a consideration of equation (22), equation (27·1) can clearly be written

$$r_4 = \frac{a}{k}\left[\frac{\lambda R_1^2 - \mu R_0 R_1}{2\lambda R_0 - \sigma R_1} - \frac{\lambda R_1^2 - \mu R_\infty R_1}{2\lambda R_\infty - \mu R_1}\right], \tag{27·2}$$

where $\lambda = 1 - \rho$, $\mu = 1 + 2\rho$, and $\sigma = 2 + \rho$.

Cole (1937) has given a simpler expression for r_4, based on the assumptions that $r_4 \gg r_1 a$ and $r_4 \gg r_2 a$. The expression is

$$\frac{\rho - \bar{\rho}}{\rho} = \frac{3r_1 a}{r_4}, \tag{28}$$

where $\bar{\rho}$ = volume concentration *if* $r_4 = \infty$. $\bar{\rho}$ is sometimes known as the 'non-conducting volume concentration', and from equation (24),

$$\bar{\rho} = 2(1 - r_1/r_0)/(2 + r_1/r_0).$$

Substituting this value in equation (28) and solving for r_4,

$$r_4 = \frac{3r_1 a\rho(2r_0 + r_1)}{\rho(2r_0 + r_1) - 2(r_0 - r_1)}. \tag{29}$$

Experimental conditions may, however, be such as to make the assumptions unwarrantable, in which case equations (27·1) and (27·2) must be used for determining r_4.

Membrane resistances are so high that their measurement presents considerable difficulties. Cells such as *Nitella*, which are normally in fresh water, may have membrane resistances of 250,000 ohm-cm^2 or more (Blinks, 1930), while the longitudinal resistance of the squid giant axon, after being immersed in sea water, is about 1000 ohm-cm^2 (Cole & Hodgkin, 1939). Cole pointed out (1937) that if the resistance of *Hipponoe* egg-cell membranes is as high as that of *Nitella*, the difference between ρ and $\bar{\rho}$ in equation (28) would be about 0·002%, which means that extremely accurate measurements of volume concentration are necessary to distinguish between a cell membrane which is assumed to have an infinite d.c. resistance and one which has a finite but very high d.c. resistance.

When such very high resistances are involved, a small error in measurement may lead to highly improbable results, such as the resistance of an egg in tap water being higher than the resistance of a glass sphere, of equal volume, in the same tap water. A convenient way of checking if measurements are of the right order and in the right sense is by comparing ρ measured, and $\bar{\rho}$ calculated on the assumption that $r_4 = \infty$, in which case $\bar{\rho} = 2(1 - r_1/r_0)/(2 + r_1/r_0)$. ρ can be measured volumetrically or by indirect methods described by Cole (1937). The larger r_4 is, the nearer ρ approaches $\bar{\rho}$. The value of ρ is *bounded below* by the value of $\bar{\rho}$. This means that unless the cell is actively *extracting* ions from the surrounding medium, a somewhat improbable situation, ρ will always be greater than $\bar{\rho}$, though, as mentioned above, the difference may be very small if r_4 is large. It automatically follows that unless the membrane resistance is so great as to be experimentally indistinguishable from a membrane with an infinite d.c. resistance, the measured volume concentration

should always be greater than the volume concentration calculated on the assumption that $r_4 = \infty$.

If the membrane resistance is finite, the previous equations for the capacitance of the membrane, in which it was assumed that $r_4 = \infty$, need modification. Instead of equation (20) being solved for $\mathbf{z}_3 = \frac{1}{j\omega c_3}$, it must be solved for $\mathbf{z}_3 = \frac{r_4}{1 + j\omega c_3 r_4}$. By simple algebraic rearrangement the capacitance per unit area is found to be

$$c_3 = \frac{-r_4 \pm \sqrt{(r_4^2 - 4aI^2)}}{2a\omega r_4 I}, \tag{30}$$

where r_4 has the value given in equation (27·1) and

$$I = \frac{9r_1^2 x\rho}{[2r(1-\rho) - r_1(2+\rho)]^2 + [2x(1-\rho)]^2}.$$

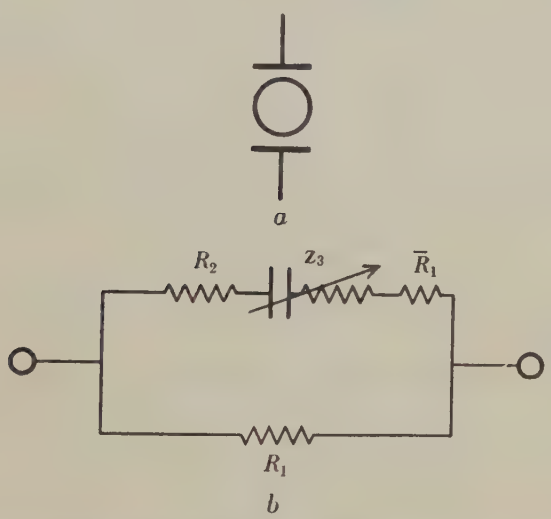

Fig. 6. *a*, spherical cell between electrodes. *b*, equivalent electrical circuit assuming $r_4 = \infty$. R_1, resistance of tap water; R_2, resistance of cell interior; $\bar{R}_1$, resistance of medium in path of current flow between electrodes and cell surface (this is small enough to be ignored); $\mathbf{z}_3$, polarization capacitance of cell surface.

This more refined method of analysis has not yet been attempted with the trout egg, though it is proposed to do it in the future.

In previous sections c_3 has been calculated from the original Maxwell equation. The cell or cells have not been considered in terms of equivalent electrical circuits and the relationships which have been derived hold good for any frequency. But it is sometimes convenient to use a different method of deriving c_3. This method is a combination of a pure electrostatic and an equivalent electrical circuit method. A spherical cell in its electrolytic cell is shown diagrammatically in Fig. 6*a*. The various cell parameters have already been defined. $\mathbf{z}_3$ is the impedance of the cell membrane. It is a polarization impedance or capacitance. r_4 is assumed to be

infinite. Fig. 6*b* shows an electrical network which is equivalent to Fig. 6*a*. By rearranging equation (19) it can be shown to be

$$\mathbf{Z}_a = \frac{2+\rho}{2(1-\rho)} r_1 \frac{\frac{1-\rho}{2+\rho} r_1 + r_2 + \mathbf{z}_3/a}{\frac{1+2\rho}{2(1-\rho)} r_1 + r_2 + \mathbf{z}_3/a}. \tag{31}$$

The impedance of system *b* is

$$\mathbf{Z}_b = R_1 \frac{R_2 + \mathbf{Z}_3}{R_1 + R_2 + \mathbf{Z}_3}. \tag{32}$$

As *b* is equivalent to *a*,

$$\mathbf{Z}_a = \mathbf{Z}_b. \tag{33}$$

At $\nu = 0$, $\mathbf{z}_3 = \infty$ and $\mathbf{Z}_3 = \infty$. Therefore

$$R_1 = \frac{2+\rho}{2(1-\rho)} r_1. \tag{34}$$

At $\nu = \infty$, $\mathbf{z}_3 = 0$ and $\mathbf{Z}_3 = 0$. Therefore

$$\frac{R_2}{R_1 + R_2} = \frac{\frac{1-\rho}{2+\rho} r_1 + r_2}{\frac{1+2\rho}{2(1-\rho)} r_1 + r_2}. \tag{35}$$

Equating the numerators and denominators of each side of this equation

$$R_2 = \gamma \left[\frac{1-\rho}{2+\rho} r_1 + r_2 \right]$$

and

$$R_1 + R_2 = \gamma \left[\frac{1+2\rho}{2(1-\rho)} r_1 + r_2 \right], \tag{36}$$

where $\gamma = \frac{2(2+\rho)^2}{9\rho}$. By algebraical rearrangement it can be shown that

$$\mathbf{Z}_3 = \frac{\gamma \mathbf{z}_3}{a}. \tag{37}$$

Therefore

$$\mathbf{z}_3 = \frac{9\rho}{(2+\rho)^2} \mathbf{Z}_3 a. \tag{38}$$

If r_4 is assumed to be infinitely great, then, as before,

$$\rho = 2 \frac{1 - r_1/r_0}{2 + r_1/r_0},$$

therefore

$$\gamma = \frac{2}{(2 + r_1/r_0)(1 - r_1/r_0)} \tag{39}$$

and

$$\mathbf{z}_3 = \frac{(2 + r_1/r_0)(1 - r_1/r_0)}{2} \mathbf{Z}_3 a. \tag{40}$$

As

$$\mathbf{Z}_b = R_1 \frac{R_2 + \mathbf{Z}_3}{R_1 + R_2 + \mathbf{Z}_3} \text{(equation (32))},$$

$$\mathbf{Z}_3 = (R_1 + R_2) \frac{\mathbf{Z}_b - \frac{R_1 R_2}{R_1 + R_2}}{R_1 - \mathbf{Z}_b}. \tag{41}$$

At $\nu=0$, $\mathbf{Z}_b$ becomes R_0 and $\mathbf{Z}_3$ becomes infinity. Substituting these values in equation (32)

$$R_0=R_1. \tag{42}$$

At $\nu=\infty$, $\mathbf{Z}_b$ becomes R_∞ and $\mathbf{Z}_3$ becomes zero. Therefore

$$R_\infty=\frac{R_1R_2}{R_1+R_2}. \tag{43}$$

Solving equation (43) for R_2 and substituting R_0 for R_1,

$$R_2=\frac{R_0R_\infty}{R_0-R_\infty}. \tag{44}$$

Substituting this value of R_2, and R_0 for R_1 in equation (41),

$$\mathbf{Z}_3=\frac{R_0^2}{R_0-R_\infty}\frac{\mathbf{Z}_b-R_\infty}{R_0-\mathbf{Z}_b}. \tag{45}$$

At any particular frequency, the electrical network b is equivalent to R_s-jX_s. R_s-jX_s is plotted in Fig. 7. In this diagram

$$\mathbf{u}=\mathbf{Z}_b-\mathbf{R}_\infty \quad \text{and} \quad \mathbf{v}=\mathbf{R}_0-\mathbf{Z}_b. \tag{46}$$

The subtractions are not scalar but vectorial. Returning to equation (45),

$$\mathbf{Z}_3=\frac{R_0^2}{R_0-R_\infty}\frac{\mathbf{u}}{\mathbf{v}}. \tag{47}$$

At a particular frequency, X_s is a minimum and $\mathbf{u}=-j\mathbf{v}$.* This is the 'characteristic frequency' $\bar{\omega}$. At $\nu=\bar{\omega}/2\pi$,

$$\bar{\mathbf{Z}}_3=-j\frac{R_0^2}{R_0-R_\infty}, \tag{48}$$

where $\bar{\mathbf{Z}}_3=\mathbf{Z}_3$ at $\bar{\omega}$. Substituting equation (48) in equation (40),

$$\bar{\mathbf{z}}_3=-j\frac{(2+r_1/r_0)(1-r_1/r_0)r_0^2a}{2(r_0-r_\infty)}, \tag{49}$$

where $\bar{\mathbf{z}}_3=\mathbf{z}_3$ at $\bar{\omega}$.

As $\bar{\mathbf{z}}_3=1/j\bar{\omega}\bar{c}_3$,

$$\bar{c}_3=\frac{2(r_0-r_\infty)}{a\bar{\omega}r_0^2(2+r_1/r_0)(1-r_1/r_0)}. \tag{49·1}$$

Equation (49·1) assumes that the membrane capacitance is 'pure', i.e. that it acts like a good wireless condenser. If, however, the capacitance is of the polarization type, equation (49) must be modified. At the characteristic frequency $\bar{\omega}$, equation (45) can be written

$$\mathbf{Z}_3=\frac{R_0^2}{R_0-R_\infty}e^{-j\phi} \tag{50}$$

$$=\frac{R_0^2}{R_0-R_\infty}(\cos\phi-j\sin\phi). \tag{50·1}$$

* This is only true when the R_s-jX_s plot is a semicircle. The more general case, when the plot is an arc of a circle, as in Fig. 7, is discussed below.

The capacitative part of this expression is

$$\frac{R_0^2}{R_0 - R_\infty} \sin\phi, \tag{50·2}$$

and the polarization capacitance will therefore be $1/\sin\phi$ of the 'pure' capacitance value. Equation (49·1) will become

$$\bar{c}_s = \frac{1}{a\bar{\omega}r_0^2} \frac{2(r_0 - r_\infty)}{(2 + r_1/r_0)(1 - r_1/r_0)\sin\phi}. \tag{51}$$

When the capacitance is 'pure', $\phi = 90°$, $\sin\phi = 1$, and equation (51) is identical with equation (49·1).

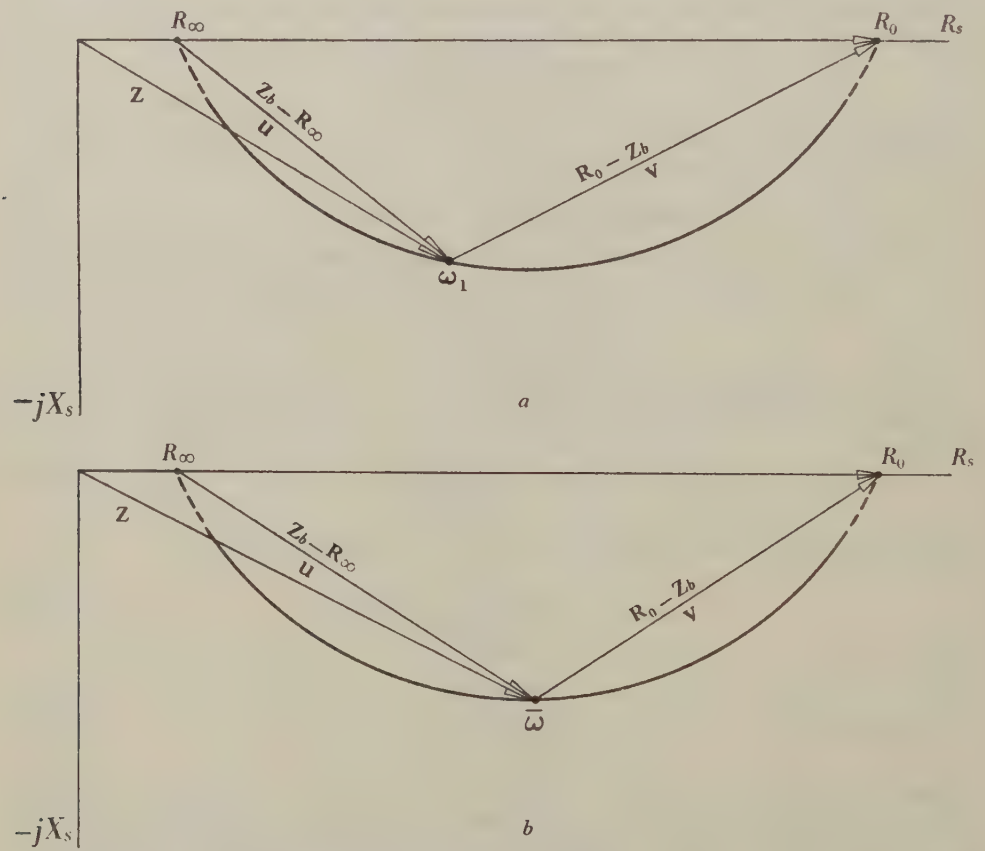

Fig. 7. *a*, the $R_s - jX_s$ plot showing impedance vector at 1 kcyc., ω_1. *b*, the $R_s - jX_s$ plot, showing impedance vector at the characteristic frequency $\bar{\omega}$, where $u = v$ and X_s is a minimum.

The characteristic frequency $\bar{\omega}$ of cells or suspensions of cells varies over wide limits according to the type of cell and the electrical properties of the surrounding medium. In those cases where the capacitance is of the polarization type, the value at $\bar{\omega}$ may not be as suitable for comparing different cells as the value at a standard frequency, for example, 1 kcyc.

Equation (51) can be converted to the capacitance at the standard frequency by means of an equation due to Cole:*

$$c_{31} = \frac{1}{a\omega_1(2+r_1/r_0)(1-r_1/r_0)r_0^2\sin\phi}\frac{2(r_0-r_\infty)}{}\left(\frac{\omega_1}{\omega}\right)^\alpha, \qquad (52)$$

where c_{31} = capacitance per unit area of membrane at 1 kcyc., $\omega_1 = 2\pi.1000$, and $\alpha = 2\phi/\pi$.

THE TROUT EGG

The trout egg is well suited to a.c. measurements. As tap water, with its relatively high resistance, is the external medium, high frequencies are not necessary to pass current through the egg surface; moreover, the egg is large, being about 5 mm. in diameter. This might give the impression that measurements of egg volumes are easy to make, but unfortunately this is not the case as will be seen later in the section entitled Sources of Error.

MATERIAL

The eggs of *Salmo irrideus* Gibbons and *Salmo fario* were used. Experiments were started about 3 hr. after the eggs had been placed in tap water. To obtain fertilized eggs, a ripe female was stripped into a bowl, and sperm, which had previously been tested for activity after dilution with water, were added to the dry eggs. Tap water was then added. A high percentage of fertilized eggs is usually obtained by this method.

METHOD

Measurements were made with an a.c. bridge which has been fully described elsewhere (Hubbard & Rothschild, 1939). The electrolytic cells were approximately cubical and made of paraffin wax, two opposite faces being platinized platinum electrodes. A glass plate was placed on top of the cell after the egg and tap water had been inserted. There was no air between the glass plate and the surface of the tap water. The dimensions of the electrolytic cells were about 7·5 mm. (length) $\times$ 7·2 mm. (width) $\times$ 7·2 mm. (height), giving a volume concentration between 0·1 and 0·2 and a cell constant of 1·08. Larger electrolytic cells, with correspondingly lower volume concentrations, were occasionally used, but as the sensitivity of this method of analysis is directly proportional to the volume concentration, the dimensions of the cell given above represent a compromise between sensitivity and the inapplicability of Maxwell's equation at high volume concentrations.

A run involves the following measurements and calculations:

(1) Determination of the cell constant k. This need not be done for each run, as it depends entirely on the geometrical characteristics of the cell.

(2) Blank run with tap water. This is to determine the specific resistance of the tap water, r_1, the resistance of the electrodes, and the capacitance of the electrodes. The values of the parallel resistance R'_p, and of the parallel capacitance C'_p,

* Private communication.

necessary to balance the electrolytic cell filled with tap water, are determined at frequencies $\nu = 0{\cdot}1 - 50$ kcyc.

(3) Egg run. This determines the values of the parallel resistance $\bar{R}_p$, and of the parallel capacitance $\bar{C}_p$, necessary to balance the electrolytic cell when it contains an egg and tap water, at frequencies $\nu = 0{\cdot}1 - 50$ kcyc.

(4) Volumetric determination of the apparent egg volume.

(5) Calculation of the resistance R_p, and capacitance C_p, due to the egg in tap water, and not including the resistance and capacitance of the electrodes, at each frequency. This is done by the relationships (Cole & Cole, 1936):

$$\bar{R}_p = R_p, \quad C_p = \bar{C}_p - \left(\frac{R'^2_p}{\bar{R}^2_p}\right) C'_p, \quad R_1 = R'_p \text{ at 50 kcyc.} \tag{53}$$

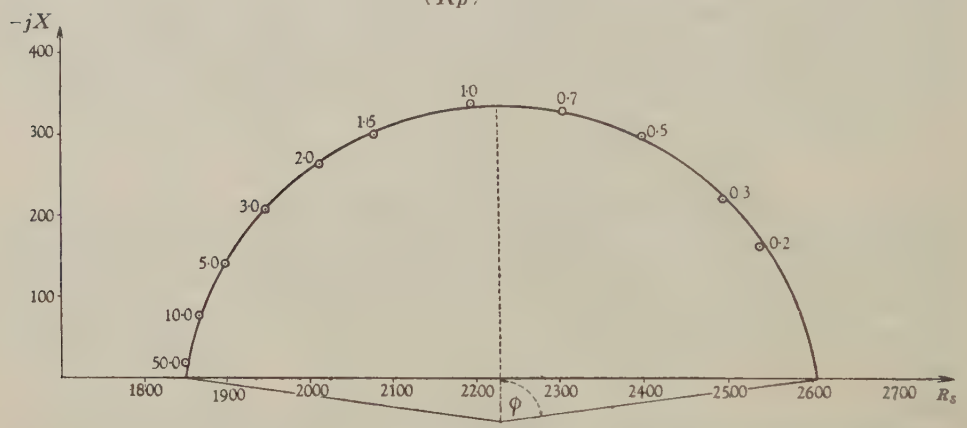

Fig. 8. $R_s - jX_s$ plot for an unfertilized trout egg in tap water. $-jX_s$ is plotted upwards. Frequencies in kilocycles. Egg no. 282246*u*.

(6) Calculation of the series resistance R_s and series reactance X_s, equivalent at each frequency to R_p and C_p, by means of the relationships

$$R_s = R_p/(1 + \omega^2 C_p^2 R_p^2), \quad X_s = \omega C_p R_p^2/(1 + \omega^2 C_p^2 R_p^2).$$

(7) Construction of circle diagram or $R_s - jX_s$ plot, and determination of R_0, R_∞, the characteristic frequency $\bar{\nu}$, and the phase angle ϕ.

(8) Calculation of c_{31}, the capacitance per unit area at 1 kcyc., by means of the equations

$$c_{31} = \frac{1}{a\omega_1 r_1^2 x} \frac{[2r(1-\rho) - r_1(2+\rho)]^2 + [2x(1-\rho)]^2}{9\rho \sin\phi}, \tag{A}$$

$$c_{31} = \frac{1}{a\omega_1} \frac{2(r_0 - r_\infty)}{(2 + r_1/r_0)(1 - r_1/r_0) r_0^2 \sin\phi} \left(\frac{\omega_1}{\bar{\omega}}\right)^\alpha. \tag{B}$$

In all calculations in this paper, the d.c. resistance of the cell membrane is assumed to be infinite; according to some calculations of Cole & Guttman (1942), Holzer (1933) obtained a value of 5000 ohm-cm^2 for the cell membrane.

A characteristic egg circle diagram is shown in Fig. 8. The phase angle between current and voltage across the membrane capacity is 82·1°. The characteristic

frequency, at which X_s is a minimum, is 0·905 kcyc. The terminals of the impedance vectors at each frequency fall accurately on an arc of a circle with centre below the R_s axis, showing that the membrane capacitance is of the polarization type.

The $R_s - jX_s$ plot for a dead egg does not give a semicircular arc because a dead egg has no capacitance and no significant resistance. The chorion is permeable to

Table 2. *Egg no.* 28, *unfertilized.* 2 *February* 1946. $T°$ C. 18·9

Volume of electrolytic cell	467 mm.³
Cell constant	1·08
Volume of egg (measured)	49·7 mm.³
Volume of egg (measured) less 100 μ shell	43·5 mm.³
R_0	2605 ohms
R_1	2278 ohms
R_∞	1850 ohms
Phase angle ϕ	82·1°
Internal resistance (ρ measured, less 100 μ shell)	192 ohm-cm.
Membrane capacitance at $\nu = 1$ kcyc. (equation (A), ρ measured less 100 μ shell)	0·54 μF.cm.$^{-2}$
Membrane capacitance at $\nu = 1$ kcyc. (equation (B), ρ calculated)	0·55 μF.cm.$^{-2}$
Characteristic frequency	0·905 kcyc.

Table 3

Unfertilized eggs

No.	Weight mg.	Vol. mm.³	$T°$ C.	$\phi°$	$\bar{\nu}$ kcyc.	c_{31} μF.cm.$^{-2}$ (A)	c_{31} μF.cm.$^{-2}$ (B)	r_2 ohm-cm.
28	55	49·7	18·9	82·1	0·905	0·54	0·55	192
30	55	50·2	18·8	84·1	0·992	0·54	0·60	188
32	60·5	54·6	19·0	81·0	0·760	0·59	0·59	172
34	61	56·8	19·2	83·0	0·750	0·62	0·67	194
36	56	50·8	19·3	83·5	0·920	0·56	0·60	170
38	56	50·5	19·4	85·0	0·900	0·48	0·47	297
	Arithmetic mean			83·1	0·871	0·56	0·58	202

Fertilized eggs

No.	Weight mg	Vol. mm.³	$T°$ C.	$\phi°$	$\bar{\nu}$ kcyc.	c_{31} μF.cm.$^{-2}$ (A)	c_{31} μF.cm.$^{-2}$ (B)	r_2 ohm-cm.
27	57	50·8	18·5	83·6	0·850	0·62	0·63	151
29	56	50·2	18·8	82·6	0·910	0·57	0·60	138
31	53	49·7	19·0	82·0	0·975	0·52	0·54	189
33	60	54·05	19·3	85·4	0·830	0·56	0·58	193
35	53	49·1	19·2	82·6	0·935	0·61	0·69	116
37	55	50·5	19·4	82·5	0·900	0·56	0·54	163·5
	Arithmetic mean			83·1	0·900	0·57	0·60	158·5

water and ions while little or nothing remains of the vitelline membrane. This fact casts some doubt on Holzer's results (referred to above) on the membrane resistance, as to measure it he cut the egg in half. This kills the egg and destroys the resistive properties of the cell membrane. In Table 2 certain egg characteristics or parameters have been calculated or measured by various alternative methods.

These experiments were originally done to see if fertilization caused any change in the cell surface, as evidenced by changes in its resistance or capacitance. Similar

experiments on other eggs have not indicated that fertilization is systematically associated with changes in capacitance, while, except in the frog's egg (Cole & Guttman, 1942), the membrane resistance of eggs seems to be almost too high to be measured. Table 3 gives the results of experiments on a batch of unfertilized and fertilized eggs. This batch was selected out of a number of other batches because of better temperature conditions. Measurements were made alternately on fertilized and unfertilized eggs to reduce the effect of variation in temperature. It will be seen that there is no significant change in capacitance after fertilization.

DISCUSSION

The main sources of error in the experiments described in this paper are:

(1) *Volume concentration.* A small error in the measurement of egg volume, and subsequent calculation of the actual egg volume causes a relatively large error in the value of a cell parameter. Suppose for example that the egg volume is 70 mm., and the electrolytic cell volume is 0·5 c.c.; therefore $\rho = 0{\cdot}14$. Let the cell constant k, R_1 and R_∞ respectively have the values 1·5, 2400 and 1700. Substituting these values in equation (22)

$$r_2 = \frac{R_1}{k}\frac{R_1(1-\rho)-R_\infty(1+2\rho)}{2R_\infty(1-\rho)-R_1(2+\rho)}, \quad r_2 = 81 \text{ ohm-cm.}$$

If an error of 2% is made in egg volume, r_2 becomes 93 ohm-cm., an error of 15%.

Although superficially the measurement of the trout-egg volume presents no difficulties, this is not in fact the case. The protoplasmic membrane is surrounded by the chorion which according to Bogucki (1930) is about 80 μ thick. Within this chorion there is a perivitelline space in which the egg proper can rotate according to its centre of gravity. The thickness of the perivitelline space is difficult to measure in living eggs and virtually impossible to measure in dead eggs. As the chorion is freely permeable to small molecules and ions, the perivitelline space must normally have the same composition as the external medium. Measurements on dead eggs show that the resistance and capacitance of the chorion is negligible if one assumes, as is usually the case, that the chorion is an 'inanimate' membrane and does not change when the egg dies. Neither the chorion nor the perivitelline space should therefore contribute to the egg volume proper, and their volumes must be subtracted from that of the total egg volume. A 100 μ shell must therefore be subtracted from the total, or apparent egg volume, but for obvious reasons a small error in shell width will cause a large error in shell volume. Equation (26) in the theoretical part of this paper shows that a small error in egg volume also has a large effect on the value of the membrane resistance.

(2) R_0. The resistance of the egg and its surrounding medium at zero frequency depends on an extrapolation. The terminals of the low-frequency impedance vectors may show deviations from the circular arc locus which is theoretically required. These deviations are probably due to electrode polarization, although a correction is included in the calculations for this effect. The value of R_0 could be checked by substituting non-polarizable silver-silver chloride electrodes for the platinized

platinum ones and by using d.c. instead of a.c. A.c. at zero frequency is of course d.c. A silver-silver chloride electrode requires a significant Cl^- concentration in the outside medium to be non-polarizable, which should therefore be changed to a solution such as a mixture of NaCl and $CaCl_2$. Although this experiment should be done, it has not been found practicable to do it this year.

(3) *Temperature.* Conductivity measurements are well known to be very sensitive to temperature changes. Thermostatting presented considerable difficulties in these experiments and was not attempted, though the room temperature was maintained as constant as possible at $17 \pm 1°$ C. The frequency of the periodic impedance changes that occur in fertilized and unfertilized trout eggs after about 8 hr. in tap water is, however, very sensitive to temperature changes, and quantitative experiments on them would require rigorous temperature control.

(4) *Geometrical and chemical heterogeneity of the egg.* The trout egg vitelline membrane is not a perfect spherical shell as it is thickened at the top to form the blastodisc. Furthermore, oily globules are distributed throughout the vitelline membrane.

(5) *Ratio of egg volume to electrolytic cell volume.* Table 1 on p. 82 shows that with glass spheres, Maxwell's equation (12) is inaccurate at high volume concentrations, as Maxwell indicated on theoretical grounds. At low volume concentrations, however, other errors creep in; a compromise is therefore necessary.

Comparisons between fertilized and unfertilized eggs would be more satisfactory if the capacitance of *one* egg could be measured before and after fertilization. This is impossible, as the act of measuring the capacitance of an unfertilized egg precludes the possibility of subsequent fertilization. Before measurements begin, the egg must have been in tap water for about 2 hr., to get osmotic equilibrium between the egg and its environment. But after being in tap water for 5 min., the chorion of the egg hardens, the micropyle, through which the spermatozoon fertilizes the egg, is occluded, and the egg is unfertilizable.

The capacitance of the trout-egg membrane is similar to that of other cell membranes. The fact that living cell membranes always seem to have a capacitance of about $1\,\mu$ F.cm.$^{-2}$ strongly suggests that there is some structural similarity between such widely different membranes as those of the Squid axon, $1{\cdot}1\,\mu$F.cm.$^{-2}$ (Cole & Curtis, 1938), unfertilized *Arbacia* eggs, $0{\cdot}73\,\mu$ F.cm.$^{-2}$ (Cole & Cole, 1936), red blood cells, $0{\cdot}81\,\mu$ F.cm.$^{-2}$ (Fricke & Curtis, 1934), and the trout egg. At present, all that can be said from the chemical point of view about cell membranes is that they are probably protein-lipoid complexes. Given a value for the capacitance, the membrane dielectric constant can be calculated if the thickness of the membrane is known; or, the thickness of the membrane can be calculated if the dielectric constant is known. As mentioned earlier in this paper, the dielectric constants of polar media depend on the polarizability of the molecules in the alternating electric field. We do not know what dipole moments the molecules in the cell membrane have, and therefore to make a guess at the membrane dielectric constant, as has sometimes been done, may result in serious errors, particularly as the tendency for non-conductors to have low dielectric constants might be counteracted by the high dielectric constants that such

compounds as glycine are known to have. Similar arguments apply with regard to the membrane resistance, when it can be measured. If the chemical composition, and therefore the specific resistance of the membrane, were known, its thickness could be calculated. If its thickness were known, its specific resistance could be calculated. In the case of the membrane resistance there are other difficulties. The membrane has holes in it which seem to permit K^+ ions to pass through more easily than Na^+ ions in the case of nerve, though this difference is less marked in the case of the trout-egg membrane (Pumphrey, 1931). What effect this heterogeneity may have on the general resistivity, or even the dielectric constant of the membrane, is difficult to estimate.

As will be seen from Table 2, there is a discrepancy between the resistance of the inside of the trout egg when measured by a.c. and when measured by Gray's more direct conductivity method. It is by no means impossible that squeezing the contents of the egg out and then measuring the conductivity might destroy or break down some colloidal structure, which would result in an increased conductivity, though some rough experiments done by Gray's method confirmed the values reported in this paper. It is very difficult to make accurate measurements of the real volume of the egg, which are required for calculation of the internal conductivity by a.c. If this difficulty is considered insurmountable, it is necessary to fall back on the assumption that the membrane has an infinite d.c. resistance. At the same time, the discrepancy would be greater if the cell membrane had a significant conductance. The resolution of this difficulty involves removing the chorion without damaging the vitelline membrane, an exceedingly delicate operation in newly laid eggs. The average resistance of the egg interior was 206 ohm-cm. (32 eggs), though low values were occasionally noted. There is not sufficient evidence to say whether the difference between the internal resistance of fertilized and unfertilized eggs is significant or not.

No physico-chemical explanation of a polarization capacitance has yet been found. A number of complicated suggestions have been made and are summarized by Cole & Cole (1941), but none are entirely successful in explaining this phenomenon, which also occurs in non-living material. Until it is better understood in relatively simple non-living systems, there is little point in speculating about its meaning in highly complex systems such as cell membranes.

SUMMARY

1. The theory of alternating current measurements as applied to biological systems is discussed and the equations for determining the resistance and capacitance of the cell membrane, and the resistance of the cell interior are deduced.

2. Maxwell's equation for the specific resistance of a random suspension of spherically packed homogeneous spheres is involved in these equations, and its applicability to single glass spheres of various diameters is established.

3. This method of analysis is applied to the egg of the trout, to examine the capacitance of its cell membrane before and after fertilization. If the membrane

resistance is assumed to be infinite, the capacitance of the cell membrane, scaled to a standard frequency of 1 kcyc., is approximately 0·57 μF.cm.$^{-2}$ in unfertilized eggs and 0·58 μF.cm.$^{-2}$ in fertilized eggs. The difference is not statistically significant.

4. The capacitative element of the cell membrane has a constant phase angle of about 83° in fertilized and unfertilized eggs, indicating dielectric loss in the membrane.

5. The characteristic frequency of fertilized and unfertilized eggs was between 0·8 and 0·9 kcyc.

6. The internal resistance of the egg varied in different batches but in general was higher than that found by Gray (90 ohm-cm.). The average value for 32 eggs was 206 ohm-cm.

7. The experiments described in this paper are neither connected with, nor affected by, the periodic impedance changes which occur at a somewhat later stage in fertilized and unfertilized trout eggs.

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